

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

United States Patent and Trademark
Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 17 February 1999 (17.02.99)	
International application No. PCT/US98/13071	Applicant's or agent's file reference NIH0082.02
International filing date (day/month/year) 25 June 1998 (25.06.98)	Priority date (day/month/year) 25 June 1997 (25.06.97)
Applicant POLYMEROPOULOS, Mihael, H. et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

25 January 1999 (25.01.99)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Athina Nickitas-Etienne

Facsimile No.: (41-22) 740.14.35

Telephone No.: (41-22) 338.83.38

2481410



From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

To:
SPENCER & FRANK
Attn. SCHNELLER, J.
Suite 300 East
1100 New York Avenue, N. W.
Washington, D.C. 20005-3955
UNITED STATES OF AMERICA

Article 19 Date Jan. 27, 1999
Date of mailing
(day, month, year) 27/11/1998

Applicant's or agent's file reference NIH0082.02	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US 98/13071	International filing date (day, month, year) 25/06/1998
Applicant THE GOVERNMENT OF THE UNITED STATES OF et al.	

1. ☒ The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

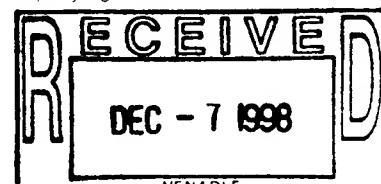
Filing of amendments and statement under Article 19

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.



2. ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040. Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Authorized officer Mireille Claudepierre
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NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.



NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged,
- (ii) the claim is cancelled,
- (iii) the claim is new,
- (iv) the claim replaces one or more claims as filed,
- (v) the claim is the result of the division of a claim as filed

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14, claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

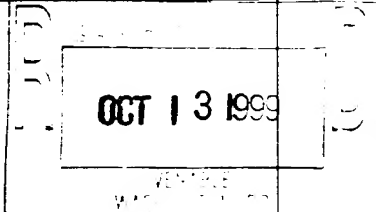
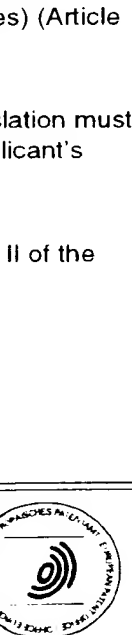
Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.



From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: SCHNELLER, John W. Venable, Baetjer, Howard and Civiletti, LLP P.O. Box 34385 Washington, DC 20043-9998 ETATS-UNIS D'AMERIQUE				PCT NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT (PCT Rule 71.1)	
		Date of mailing (day/month/year)		05.10.99	
Applicant's or agent's file reference <u>NIH0082.02</u> 31978-141234		IMPORTANT NOTIFICATION			
International application No. PCT/US98/13071		International filing date (day/month/year) 25/06/1998		Priority date (day/month/year) 25/06/1997	
Applicant THE GOVERNMENT OF THE UNITED STATES... et al.		NO Due Date m.m. 			


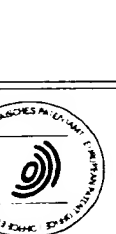
1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx. 523656 epmu d Fax: +49 89 2399 - 4465		Authorized officer Schou, S Tel. +49 89 2399-8062		
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PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference NIH0082.02	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/US 98/ 13071	International filing date (day/month/year) 25/06/1998	(Earliest) Priority Date (day/month/year) 25/06/1997
Applicant THE GOVERNMENT OF THE UNITED STATES OF et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. ☐ Certain claims were found unsearchable (see Box I).

2. ☐ Unity of invention is lacking (see Box II).

3. ☒ The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing

☒ filed with the international application.

☐ furnished by the applicant separately from the international application.

☐ but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

☐ Transcribed by this Authority

4. With regard to the **title**, ☒ the text is approved as submitted by the applicant

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

Figure No. 1 ☒ as suggested by the applicant.

☐ None of the figures.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.



INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/13071

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/47 C12N15/11 C07K16/18 A61K48/00
 C12Q1/68 G01N33/68 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K C12Q G01N A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	SCHAPIRA A. H.: "Pathogenesis of Parkinson's disease." BAILLERES CLINICAL NEUROLOGY, vol. 6, no. 1, April 1997, pages 15-36, XP002083889	1-23, 57-61, 74
Y	see page 17, paragraph 2 see abstract	24-56, 62-73
Y	US 5 494 794 A (WALLACE DOUGLAS C) 27 February 1996 see the whole document --- -/-	24-56, 62-73

☒ Further documents are listed in the continuation of box C☒ Patent family members are listed in annex

Special categories of cited documents

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

Date of the actual completion of the international search

10 November 1998

Date of mailing of the international search report

27/11/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel: (+31-70) 340-2040, Tx: 31 651 epo.nl
 Fax: (+31-70) 340-3016

Authorized officer

Mandl, B



INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/13071

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication where appropriate of the relevant passages	Relevant to claim No.
A	JAKES R. ET AL.: "Identification of two distinct synucleins from human brain." FEBS LETTERS, vol. 345, 1994, pages 27-32, XP002078475 cited in the application & UEDA K. ET AL.: "Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease." PROC. NATL. ACAD. SCI. USA, vol. 90, 1993, pages 11282-11286, see figure 2 ---	1-74
A	CHEN X. ET AL.: "The human NACP/alpha-synuclein gene: chromosome assignment to 4q21.3-q22 and TaqI RFLP analysis." GENOMICS, vol. 26, no. 2, 1995, pages 425-427, XP002083890 cited in the application ---	1-74
A	POLYMEROPOULOS M. H. ET AL.: "Mapping of a gene for Parkinson's disease to chromosome 4q21-q23." SCIENCE, vol. 274, 1996, pages 1197-1199, XP002083891 cited in the application see the whole document ---	1-74
A	MAROTEAUX L. AND SCHELLER R. H.: "The rat brain synucleins: family of proteins transiently associated with neuronal membrane." MOLECULAR BRAIN RESEARCH, vol. 11, 1991, pages 335-343, XP002083892 cited in the application see figure 1 ---	1-74
P.X	NUSSBAUM R. L. AND POLYMEROPOULOS M. H.: "Genetics of Parkinson's disease." HUMAN MOLECULAR GENETICS, vol. 6, no. 10, 1997, pages 1687-1691, XP002083893 see the whole document ---	1-74
P.X	GOEDERT M.: "The awakening of alpha-synuclein." NATURE, vol. 388, 17 July 1997, pages 232-233, XP002083894 see the whole document ---	1-74

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/13071

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication where appropriate of the relevant passages	Relevant to claim No.
P.X	<p>POLYMEROPOULOS M. H. ET AL.: "Mutation in the alpha-synuclein gene identified in families with Parkinson's disease." SCIENCE, vol. 276, 27 June 1997, pages 2045-2047. XP002083895 see the whole document -----</p>	1-74



INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/13071

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5494794 A	27-02-1996	WO 9409162 A	28-04-1994



PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference NIH0082.02	<div style="display: flex; justify-content: space-between;"> <div> FOR FURTHER ACTION </div> <div> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) </div> </div>	
International application No. PCT/US98/13071	International filing date (day/month/year) 25/06/1998	Priority date (day/month/year) 25/06/1997
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant THE GOVERNMENT OF THE UNITED STATES... et al.		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 8 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 18 sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input checked="" type="checkbox"/> Priority III <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input checked="" type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application 		
Date of submission of the demand 25/01/1999	Date of completion of this report <div style="text-align: right; font-size: 1.2em;">05. 10. 99</div>	
Name and mailing address of the international preliminary examining authority: <div style="display: flex; align-items: center;"> <div> European Patent Office D-80298 Munich Tel: +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 </div> </div>	Authorized officer Herrmann, K Telephone No. +49 89 2399 2670	





**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US98/13071

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-49	as originally filed		
50-65	as received on	17/02/1999 with letter of	10/02/1999

Claims, No.:

1-48,68 (part), 69-74	as originally filed	
49-56,62-67, 68 (part)	with telefax of	01/07/1999

Drawings, sheets:

1/14-14/14	as originally filed
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2. The amendments have resulted in the cancellation of:

<input checked="" type="checkbox"/> the description,	pages:	66-68
<input checked="" type="checkbox"/> the claims,	Nos.:	57-61
<input type="checkbox"/> the drawings,	sheets:	

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US98/13071

- ☐ copy of the earlier application whose priority has been claimed.
- ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 7, 12-14, 24, 25, 29, 33-36, 38-41, 44-46, 50, 53, 64-74.

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 7, 12-14, 24, 25, 29, 33-36, 38-41, 44-46, 50, 53, 64-74 are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☐ no international search report has been established for the said claims Nos. .



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US98/13071

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4-6, 8-11, 15, 16, 19, 21, 22, 26-28, 30-32, 37, 42, 43, 47-49, 51, 52, 54-56, 62, 63
	No:	Claims	1-3, 17, 18, 20, 23
Inventive step (IS)	Yes:	Claims	4-6, 8, 9, 15, 16, 19, 21, 22, 26-28, 30-32, 37, 42, 43, 47-49, 51, 52, 54, 55, 62, 63
	No:	Claims	1-3, 10, 11, 17, 18, 20, 23, 56
Industrial applicability (IA)	Yes:	Claims	1-6, 8-11, 15-23, 26-28, 30-32, 37, 42, 43, 47-49, 51, 52, 54-63
	No:	Claims	

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet



Citations

- 1 The documents mentioned in this international preliminary examination report (IPER) are numbered as in the international search report dated 10.11.98, i.e. D1 corresponds to the first document of the search report etc.
- 2 A letter of the Assistant Journals Publisher for Harcourt Brace and Company who works on the publication of the "Baillière's Clinical Neurology" series dated 08.03.99 has been filed on 01.07.99. According to said letter the article by A.H.V. Schapira (**D1**) has not been printed and published and thus made available to the public before November 1997 (see **item II**, *infra*).

Re ITEM I (Basis of the opinion)

- 1 The amended description pages 50-65 filed with letter dated 10.02.99 can be regarded as meeting the requirements of Art. 34(2)(b) PCT.
- 2 Claims 49-56, 62-67 and 68 (partially) filed with telefax of 01.07.99 can be regarded as meeting the requirements of Art. 34(2)(b) PCT.

Re ITEM II (Priority)

Since the priority document pertaining to the present application is not yet available to the IPEA, this IPER has been drawn up considering the priority date (25.06.97) as valid. Documents **D1** and **D7-D9** have been published between the priority date and the filing date of the present application. Thus, said documents do not constitute prior art in the meaning of Rule 64(1)(b) PCT. However, if it turns out that the effective date of the claimed subject-matter is not the priority date then **D1** and **D7-D9** will become relevant to assess whether the present application satisfies the criteria set forth in Art. 33(2) and (3) PCT.

Re ITEM III (Non-establishment of opinion)

- 1 The invention of present application is a specific mutation in a specific human synuclein gene (G209A in human alpha-synuclein) which leads to an A53T



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US98/13071

change in the corresponding human alpha-synuclein protein. This specific molecular alteration is said to be causative for Parkinson's disease in at least four families. (see p. 1 of present description).

- 2 Claims 7, 12-14, 24, 25, 29, 33-36, 38-41, 44-46, 50, 53 and 64-73 do clearly not comply with the requirements of Art. 6 PCT. Said claims do not contain the essential technical features needed to define the invention. Claim 74 contains no technical features. Thus, a meaningful examination could not be carried out for the subject-matter of said claims.

Consequently, international preliminary examination has been restricted to the subject-matter of claims 1-6, 8-11, 15-23, 26-28, 30-32, 37, 42, 43, 47-49, 51, 52 and 54-63.

Re ITEM V (Novelty, inventive step, industrial applicability)

1 Novelty (Art. 33(2) PCT)

- 1.1 The subject-matter of claims 4-6, 8-11, 15, 16, 19, 21, 22, 26-28, 30-32, 37, 42, 43, 47-49, 51, 52, 54-56, 62 and 63 has not been made available to the public by any of the available prior art documents and can therefore be regarded as novel.
- 1.2 Due to the very open claim language recited in claims 1-3, 17, 18 and 20 the subject-matter of said claims does not meet the requirements of Art. 33(2) and 33(3) PCT.

The skilled person very well understands what is meant by the terms "mutated" (e.g. claim 1), "homologue" (e.g. claim 1), "portion" and "fragment" (e.g. claim 20). A "mutation", for instance, can mean the deletion of a whole gene or any "portion" of a gene, a "fragment" of DNA can be as small as two or three nucleic acid molecules, a "homologue" can evolve through "mutations" occurring in a duplicated parent gene.

Said terms therefore render the scope of claims 1-3, 17, 18 and 20 so broad that any prior art nucleotide sequence encoding a synuclein protein deprives said



claims of novelty (e.g. human alpha- and beta-synuclein disclosed in **D3**, Fig. 2 and 3; see **D6**, Fig. 1).

- 1.3 In **D3** (Fig. 4) antiserum against alpha- and beta-synuclein is disclosed. **D6** discloses an antibody against rat synuclein (**D6**, abstract and Fig. 6). The IPEA is of the opinion that the antibodies disclosed in **D3** and **D6** bind to the peptides which fall under the broad scope of claim 17. The subject-matter of claim 23 can therefore not be regarded as novel over the prior art (Art. 33(2) and (3) PCT).

2 Inventive step (Art. 33(3) PCT)

- 2.1 The subject-matter of claims 4-6, 8, 9, 15, 16, 19, 21, 22, 26-28, 30-32, 37, 42, 43, 47-49, 51, 52, 54, 55, 62 and 63 cannot be derived from the available prior art in an obvious manner and therefore complies with the requirements of Art. 33(3) PCT.
- 2.2 The subject-matter of claims 10, 11 and 56 does not contribute to an inventive solution of an unexpected technical problem. Said claims contain subject-matter which is considered merely an obvious modification to a person skilled in the art.

3 Industrial applicability (Art. 33(4) PCT)

Claims 1-6, 8-11, 15-23, 26-28, 30-32, 37, 42, 43, 47-49, 51, 52 and 54-63 meet the criteria as set forth by Art. 33(4) PCT.

Re ITEM VII (Certain defects in the international application)

The present application contains such a high number of independent claims (21 out of 74!) that the application as a whole lacks conciseness (Rule 6.1(a) PCT). Independent claims which are directed to the same category (or merely worded differently) shall be made dependent upon each other to meet the requirements of Art. 6 PCT in combination with Rule 6.4 PCT.

For example, independent claims 50-56 (which *refer* to claims directed to a different entity, viz. oligonucleotides) are all directed to "a diagnostic kit".



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

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Furthermore, the fact that numerous independent claims do not contain the essential technical features needed to define the invention (G209A mutation in the human alpha-synuclein gene, A53T change in the human alpha-synuclein protein), would justify a lack of unity objection under Art. 13 PCT.

Re ITEM VIII (Certain observations on the international application)

Rule 6.3(a) PCT requires that the matter for which protection is sought be defined in terms of technical features of the invention (also cf. PCT Guidelines III-4.4, as in force from 09.10.98). The nucleic acid/peptide of e.g. claims 1, 7 and 17 is a chemical compound which can be clearly and unambiguously defined by its chemical structure, i.e., its nucleic/amino acid sequence (reference to the appropriate SEQ ID NO not given in said claims).



SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: The Government of the United States of
America as represented by the Department of Health
and Human Services at the National Institutes of Health
(B) STREET: 6011 Executive Blvd., Suite 325
(C) CITY: Rockville
(D) STATE: Maryland
(E) COUNTRY: USA
(F) ZIP: 20852

(ii) TITLE OF INVENTION: Cloning of a gene mutation for
Parkinson's disease

(iii) NUMBER OF SEQUENCES: 19

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.01

(v) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT/US98 13071
(B) FILING DATE: 25-JUN-1998

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 216 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(B) CLONE: alpha synuclein gene exon 4 region

(vi) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4
(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

GCTAATCAGC AATTTAAGGC TAGCTTGAGA CTTATGTCTT GAATTTGTTT TTGTAGGCTC      60
CAAAACCAAG GAGGGAGTGG TSCATGGTGT GACAACAGGT AAGCTCCATT GTGCTTATAT      120

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CAAAGATGAT ATNTAAAGTA TCTAGTGAAT AGTGTGGCCC AGTATCAAGA TTTCTATGAA 180
ATTGTAAAAC AATCACTGAG CATCTAAGAA CATATC 216

(2) INFORMATION FOR SEQ ID NO:2:

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(11) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer #3"

(111) HYPOTHETICAL: NO

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GCTAATCAGC AATTAGGCT AG 22

(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(11) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer #13"

(111) HYPOTHETICAL: NO

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTATACAAGA ATCTACGAST C 21

(2) INFORMATION FOR SEQ ID NO:4:

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(11) MOLECULE TYPE: peptide

(111) HYPOTHETICAL: NO

(112) ANTI-SENSE: NO

(113) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens
(C) INDIVIDUAL ISOLATE: Swiss-Prot P37840



(viii) IMMEDIATE SOURCE:

(B) CLONE: alpha synuclein protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
1           5           10           15
Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
20           25           30
Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
35           40           45
Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
50           55           60
Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
65           70           75           80
Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys
85           90           95
Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile
100          105          110
Leu Glu Asp Met Pro Val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro
115          120          125
Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
130          135          140

```

(xii) INFORMATION FOR SEQ ID NO:5:

i. SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 140 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

ii. MOLECULE TYPE: peptide

iii. HYPOTHETICAL: NO

iv. ANTI-SENSE: NO

vi. ORIGINAL SOURCE:

- (A) ORGANISM: Rattus norvegicus
- (C) INDIVIDUAL ISOLATE: Swiss-Prot P37377

(viii) IMMEDIATE SOURCE:

(B) CLONE: alpha synuclein protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
1           5           10           15

```



Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
 20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45

Val His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
 50 55 60

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
 65 70 75 80

Thr Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Phe Val Lys
 85 90 95

Lys Asp Gln Met Gly Lys Gly Glu Glu Gly Tyr Pro Gln Glu Gly Ile
 100 105 110

Leu Glu Asp Met Pro Val Asp Pro Ser Ser Glu Ala Tyr Glu Met Pro
 115 120 125

Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
 130 135 140

2 INFORMATION FOR SEQ ID NO:6:

2.1 SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 134 amino acids
- (B) TYPE: amino acid
- (C) TOPOLOGY: linear

2.2 MOLECULE TYPE: peptide

2.3 HYPOTHETICAL: NO

2.4 ANTI-SENSE: NO

2.5 ORIGINAL SOURCE:

- (A) ORGANISM: Bos taurus
- (C) INDIVIDUAL ISOLATE: Swiss-Prot P03567

2.6 IMMEDIATE SOURCE:

- (B) CLONE: alpha synuclein protein

3.1 SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asp Val Phe Met Lys Gly Leu Ser Met Ala Lys Glu Gly Val Val
 1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Thr Glu Ala Ala Glu Lys
 20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45

Val Gln Gly Val Ala Ser Val Ala Glu Lys Thr Lys Glu Gln Ala Ser
 50 55 60



His Leu Gly Gly Ala Val Phe Ser Gly Ala Gly Asn Ile Ala Ala Ala
 65 70 75 80
 Thr Gly Leu Val Lys Lys Glu Glu Phe Pro Thr Asp Leu Lys Pro Glu
 85 90 95
 Glu Val Ala Gln Glu Ala Ala Glu Glu Pro Leu Ile Glu Pro Leu Met
 100 105 110
 Glu Pro Glu Gly Glu Ser Tyr Glu Glu Gln Pro Gln Glu Glu Tyr Gln
 115 120 125
 Glu Tyr Glu Pro Glu Ala
 130

1. INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 142 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Serinus canaria*
 (C) INDIVIDUAL ISOLATE: genbank U33860
- (vii) IMMEDIATE SOURCE:
 (B) CLONE: alpha synuclein homologue

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Val Val Ala
 1 5 10 15
 Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys Thr
 20 25 30
 Lys Glu Gly Val Leu Tyr Val Gly Ser Arg Thr Lys Glu Gly Val Val
 35 40 45
 His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Ser Asn
 50 55 60
 Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys Thr
 65 70 75 80
 Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Leu Val Lys Lys
 85 90 95
 Asp Gln Leu Ala Lys Gln Asn Glu Glu Gly Phe Leu Gln Glu Gly Met
 100 105 110



Val Asn Asn Thr Gly Ala Ala Val Asp Pro Asp Asn Glu Ala Tyr Glu
 115 120 125

Met Pro Pro Glu Glu Glu Tyr Gln Asp Tyr Glu Pro Glu Ala
 130 135 140

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 143 amino acids
 (B) TYPE: amino acid
 (C) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) ORIGINAL SOURCE:
 (A) ORGANISM: Torpedo californica
 (C) INDIVIDUAL ISOLATE: Swiss-Prot P37379
- (vi) IMMEDIATE SOURCE:
 (B) CLONE: alpha synuclein homologue

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Asp Val Leu Lys Lys Gly Phe Ser Phe Ala Lys Glu Gly Val Val
 1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Gln Asp Ala Ala Glu Lys
 20 25 30

Thr Lys Gln Gly Val Gln Asp Ala Ala Glu Lys Thr Lys Glu Gly Val
 35 40 45

Met Tyr Val Gly Thr Lys Thr Lys Glu Gly Val Val Gln Ser Val Asn
 50 55 60

Thr Val Thr Glu Lys Thr Lys Glu Gln Ala Asn Val Val Gly Gly Ala
 65 70 75 80

Val Val Ala Gly Val Asn Thr Val Ala Ser Lys Thr Val Glu Gly Val
 85 90 95

Glu Asn Val Ala Ala Ala Ser Gly Val Val Lys Leu Asp Glu His Gly
 100 105 110

Arg Glu Ile Pro Ala Glu Gln Val Ala Glu Gly Lys Gln Thr Thr Gln
 115 120 125

Glu Pro Leu Val Glu Ala Thr Glu Ala Thr Gln Glu Thr Gly Lys
 130 135 140

(2) INFORMATION FOR SEQ ID NO:9:

1. SEQUENCE CHARACTERISTICS:



- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer #1F"

- (iii) HYPOTHETICAL: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACGACAGTGT GTGTAAAG

19

- (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer #13R"

- (iii) HYPOTHETICAL: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AACATCTCTC AGCAGATCTC

20

- (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2804 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: BAC clone 139A20 Human Beta Synuclein Gene

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCGCCGCAGC CGCCGCTCCA TCCCCAGCCC CGGCCCGCA TCCGGTTTGG AAGGGGGCTG

60



CAAGTTTGCA	AGGGGCCCCG	GANAAAAAANC	GAGCAGTGGC	CCTTCCCCCG	TCCJAGGGT	120
TTCAAGGGAC	GCTAGGANTN	TCCGCGGGCC	TGGAGGTTTG	CAGTGGGGAG	TGGGGTGAGA	180
TGGGGGGAAA	GCGGGAGGGG	GCTCAGGGTC	CAGAAGGGCN	CCGCGGTCTC	GGGASTAGGG	240
GGGCATNTGC	GTCCCBGGG	AGGGGCTGGG	GTGAGASTGC	GGGGCCAGTG	CACCGSTGCC	300
CCTGTATCGC	CCTCCCCAGG	CCGCCAGGAT	GGACGTGTTC	ATGAAGGGCC	TGTCCATGGC	360
CAAGGAGGGC	GTGTGTGGCA	CCGCCGAGAA	AACCAAGCAG	GGGGTCACCG	AGGGGGGGGA	420
GAAGACCAAG	GAGGGGCTCC	TCTACGTGGG	TGGGCGGGGG	GCGGGGTTTT	TGGGGCTGCA	480
GGGCTGGGGG	TCCCGCTAGA	GTGTGAGCT	CCGGCGGGGT	TCCGGGGAGG	GTGGTTCTGC	540
GCAAGATAAT	ATNANTCAGC	AGATGGGGCN	AGGTCANCAN	GGGTGATAAG	GGACATACCC	600
ANCCCATAGA	ANCCTGGGTG	TGTATCGGGA	AATGGGGACA	CGGGGGGGGG	TGATGAGGTG	660
GGGGGCTCCA	NCTGAAAGGC	CAGGGACCCAN	TGCANTNATA	AAANACACAC	NCCTCCTTTT	720
TCTTATCTTT	TTTACCATTA	TTAATAGTTA	TCTGGTGTTC	AACACTTTCT	GTATGCCAAG	780
TACTGGGTAA	AATGTGATAA	CATCATTTC	CTCATGTAAT	GCTTCCGGCC	ATTCTACAGG	840
TGAGGGAAAC	TGGGCTTCCC	ATTGCTAGNT	AAATTTTAGG	TTCAGAAAGG	CTTGAATTGA	900
ATGTGAGTTC	AGCCAAATTC	TTAGTGGTGG	AACCAAACTG	AGTTCCATCC	GTGAAACGGG	960
GACATAAACA	GCACCCGCTT	CCCAGGGCTG	GGGAAAAGTG	AAATGCAGCG	GGGCAGGCAG	1020
AGGACTTGAC	ACAGCACTGG	CCCTCAGCCA	ACATCCACTA	GAGGGGTGGG	GTATCGCATC	1080
AGGTGGGAGA	GAACTGCAAC	CCTTGCAGAC	AGAGGTGTGG	GGCCCAGTGC	AGTCATAAGA	1140
CGGGGGTTAA	CATGGGGGTG	CAGGTTGTAG	GATNTGGGGA	CCCAAGGAGG	CAGTGAAGGG	1200
GCCAGGATGC	CCACTCTGTA	ATCACCATGC	TGTGCTGGAG	TTTCTGTTC	CTCAGCGCAG	1260
AGTCCTTAAA	TGTGCCGCTT	TTTCTNCCCT	GCAGGAAGCA	AGACCCGAGA	AGGTGTGGTA	1320
CAAGGTGTGG	CTTCAGGTAC	TAGCCCAGCC	CTGGCACCCAG	CCCTTCTCTC	AMTTAGGGGG	1380
ATGATCTGGC	CGGGAACCCAG	AGGGCGGGGG	CGGGGGAGAC	TCCCAAGGCT	TCTGGGGGAA	1440
TGCTCCGTGG	GGAGGGCAGG	CCCTGGGATA	CTACAAGGCA	GGGCATCGGT	GTTCGCCGCT	1500
GGCTCCCAAA	CGCCTTCCCTC	AACCCCTCC	CTGCTCCAGT	GGCTGAAAAA	ACCAAGGAAC	1560
AGGCCTCACA	TCTGGGAGGA	GCTGTGTTCT	CTGGGGCAGG	GAAACATGCA	GCAGCCACAG	1620
GACTGGTGAA	GAGGGAGGAA	TTCCCTACTG	ATCTGAAGGT	AAGCGATCCT	TCTGACCCGC	1680
ACATGCAGGC	AAACACACAC	ACACACACAC	ACACACACCN	GGCAGACAAA	TAAACCTGTC	1740
ACCATCCCCG	CCCCCTAAT	CCTGCCACCA	GCTTGGAACA	CAAGCCACTT	TGCCTCCCAT	1800
CCTGCGGGCC	CCTGCTAGAC	TCAGCTCAGA	ATGCATCTGA	ATAANGGCT	GCATGGGTGT	1860



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GACGCTCCCG GTGATGGGGA CCCAGACCTG GCTGTCTGCG TGTATCCTGC TTGCCAGCGT 1920
GACCCATATG ACTTCTGGCC ACGTCTGCAT GTGTCAATGA TTGTTTATTC ATTTCTTTTC 1980
ATTCAACAAA TATCCATGCC ANANCCAGCC CTGTCCTTGA GCTTCCAGNT CCGTTTCAGC 2040
CNAGGGGAGC NTGAGGGTTA TTTTGGGGT CCGGATGCCC AGCAGAGAGC CTGACACAAA 2100
GGATGAGGCA TAAGCTGGTG ANTGASTATC CAAATGGTGG AAGTGTGGAG GNTGCCAGGC 2160
ATTGGGGGAG CGGCCTGGAG AGCCAGCTCC CCAATCCATG CTGCCACTTC AACTGTGATT 2220
CGGGGGAATT TCCCGCTTCA CTTCCATCCC ACTTCCAAGG CACTCCAAAT AAATAACTGA 2280
ATTAGAAATT ATGCTTCTTT TCCCAATCCA CCTAGCCTT CCGCACTCCA ACCCACTCCA 2340
AGCTTACCAC TGTGGGAATT TGGGGGGCAT CTTGGCTGTC CTCACGAGTC CTGAGCTTTT 2400
CTGCCACAG CCAGAGGAAG TGGCCAGGA AGCTCTGAA GAACCACTGA TTGAGGCGCT 2460
GATGGAGCCA GAAGGGGAGA GTTATGAGGA CCCACCCAG GAGGAATATC AGGAGTATGA 2520
GCCAGAGGCG TAGGGGCCCA GSAGAGGCC CACCAGCAGC ACAATTCTGT CCGTGTCCCT 2580
GGCCCGCCCC CCAGAGCCAG GGTGTCTCTT AACTCTCTTC TCCCAATCA CGAGATCTTC 2640
CTTCCTCTCT GAGGCAACCC CTTGGAGCC TGTGTTAGTG TCTGTCCATC TGTGTGTCTT 2700
ACCCGCCCCC GTCCACCCC GGGGCATGGA CAGGCCCAGG GTTCCGGTCC CGGCTGGGAG 2760
CCTCGCCCCC CCAGTGTTCG CTCCTCCAT CCAGCGTCTG CGCG 2804

```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 223 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

- (B) CLONE: BAC clone 174P13 Human Gamma Synuclein Gene,
5' end

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

AGGGAGATCC AGCTCCCTCC TGCCTGCAGC AGCACAACCC TGCACACCCA CCATGGATGT 60
CTTCAAGAAG GGCTTCTCCA TCGCCAAGGA GGGNGTGSTG GGTGCGGTGG AAAAGACCAA 120
GCAGGGGGTG ACGGAAGCAG CTGAGAAGAC CAAGGAGGGG GTCATGTATG TGGGATTACA 180

```



TTTTTTTTTTT AAAGAAAGAA TAAATTAATT GTGATTAAAG TTG

223

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 677 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

- (B) CLONE: BAC clone 174P13 Human Gamma Synuclein Gene,
3' end

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTTTTTNAGG	GGGGAACA	GGGAATANAA	AAANANGGGG	GGGGGTTTTT	NNGGGGGGGG	60
GGGGAAAANG	GTNNGGGGN	NAACCNAAA	AAANNCCNAN	GGGGGGGGNN	ANTNAANTTT	120
TGGGAACCCA	AAGCCNAGG	AGGATTTTTN	GTNAANAACG	TNACCTCNAG	TGGGNCGAGG	180
AAGACCAAGG	AAANGCCCAA	CNCGGTTGAN	CGAGGCTGTG	GTGAACANCG	TNCAACNCTG	240
TGCCCNCCAA	NANCGTGGAG	GNGGCGGAGA	ACATCSCGGT	CACCTCCGGG	GTGGTGCGCM	300
AGGAGGACTT	GAGGCCATCT	CCCCCCMAC	AGGAGGGTGT	GGCATCCMAA	GAPAAAGAGG	360
AAGTGGCAGA	GGAGGCCAG	AGTGGGGGAR	ACTAGAGGGC	TACAGGCCAG	CGTGGATGAC	420
CTGAAGAGCG	CTCCTCTGCC	TTGGACACCA	TCCCCTCCTA	GCACAAGGAG	TGCCCCGCTT	480
GAGTGACATG	CGGCTGCCCA	CGCTCCTGCC	CTCGTCTTCC	TGGCCACCCT	TGGCCTGTCC	540
ACCTGTGCTG	CTGCACCAAC	CTCACTGCCC	TCCCTCGGCC	CCACCCACCC	TCTGGTCCTT	600
CTGACCCAC	TTATGCTGCT	GTGAATTTTT	TTTTTAAATG	ATTCCAAATA	AAACTTGAGC	660
CCACTCCAAA	AAAAAAA					677

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1181 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO



(iv) ANTI-SENSE: NO

vii IMMEDIATE SOURCE:

(B) CLONE: human alpha synuclein gene, exons 1 and 2 plus flanking intron sequences

viii POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4
(B) MAP POSITION: 4q21-q22

xii SEQUENCE DESCRIPTION: SEQ ID NO:14:

```

AAATTTCAGCG ATGCGAGGGG AAAGCGCTCT CCGCTCTGG GTCTGATTA TTTTGGGGG      60
CTGCGCTGTCT CTTCCAGCAG CTCCCGAAGG CATAGGCTCT GCGCTTGCTG CTGAGCGCTC    120
AGGCGCTCGN TCTCCAGGN CCACTCTGAC GAGGGGTAGG GGGTGGTCCC CNGGAGGAGC    180
CAGAGGAAGG GCGGCGACAA GAAGGAGAGG GAAGGGGAAA GAGGAAGAGG CATCATCCCT    240
AGCGCAAGCG CTCCCGATCT CCACAAGAGT GCTCGTGACC CTAAACTTAA CGTGAGGCGC    300
AAAAGCGGCG CAACCTTTTC CCGCTTGN CCAGCGAGG GGTGAGATT GATGGCTCAC    360
CGCGCGCGCG CTGCTGCATC CCGATCCGAG ATAGGGACGA GGAGCAGCT GCAGCGAAG    420
CAGCGAGCGC CGGGAGAGG GCGGGCAGAA CGCTGACAA ATCAGCGGTG GGGGCGGAGA    480
GCGGAGGAGA AGGAGAAGGA GGAGGACTAG GAGGAGGAGG ACGGCGACGA CCAGAGGGGG    540
CCCAAGAGAG GGGGCGAGCG ACCGAGCGCC GCGACGCGAA GTGAGGTGCG TCGGGGCTCA    600
GCGCAGACCC CGGCGCGGCC CTTCTCTGAG GCGTCTGGG CGCTCCCTCA CGCTTTGCTT    660
TCAAGCCTTC TCGCTTTCCA CCTCTGTGAG CGGAGAACTG GGAGTGGCCA TTGAGAGACA    720
GCTTAGCGGG TTTGCTTCCC ACTCCCGCAG CTTGCGGTG CCGGCTCACA GCGGCTCTCT    780
CTGGCGACAG TCCCCCGCGG GTGCGGCTCC GCGCTTCTG TCGGCTCTCT TCGCTTCTCT    840
TTTCTATTA AATATTATT GGGAAATTGT TAAATTTTT TTTTAAAAA AGAGAGAGGC    900
CNGGAGGAGT CGGAGTTGTG GAGAAGCAGA GGGACTCAGG TAAGTACCTG TCGATCTAAA    960
CGGGNGTCTT TGGAAATCTT GGAGAAGCGC GGATGGAGAG GAATGGTCTT GGGNAGCGGG   1020
AGGGGGTCTG GCTGCGATGA GGACCGCTGG GCCAGGTCTC TGGGAGGTGA CTACTTGTCT   1080
TTTGGGGAGC CTAAGGAAAG AGACTTBACC TGGCTTTCTG CTTGCTTCTG ATATTCCCTT   1140
CTCCACCAAG GCTGAGAGNT TAGGCTGCTT CTCCGGGATC C                               1181

```

(c) INFORMATION FOR SEQ ID NO:15:

i SEQUENCE CHARACTERISTICS:

(A) LENGTH: 536 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double



(D) TOPOLOGY: linear

(II) MOLECULE TYPE: DNA (genomic)

(III) HYPOTHETICAL: NO

(IV) ANTI-SENSE: NO

(VII) IMMEDIATE SOURCE:

(B) CLONE: human alpha synuclein gene exon 3 plus
flanking intron sequences

(VIII) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4
(B) MAP POSITION: 4q21-q22

(X) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```

CTTAAAGAG TGTACACTT TGGAGGGTTT CTCATGATTT TTCAGTGTTC TTTGTTTATT      60
TTTCCCCGAA AGTTCTCATT CAAAGTGTAT TTTATGTTTT CCAGTGTGGT GTAAAGAAAT      120
TCATTAGTCA TGGATGTATT CATGAAAGGA CTTTCAPAGG CCAAGGAGGG AGTTGTGGGT      180
GCTGCTGAGA AAACCAACA GGGTGTGCA GAAGCAGCAG GAAAGACAAA AGAGGGTGTG      240
CTCTATGTAG GTAGGTAAAG TCCAAATGTC AGTTTGGTGC TTGTTTCATGA GTGATGGGT      300
AGGATAACAA TACTCTAAAT GCTGGTAGTT CTCTCTCTTG ATTCATTTTT GCATCATTCG      360
TTGTCAAAAA GGTGGACTGA GTCAGAGGTA TGTGTAGGTA GGTGAATGTG AACGTGTGTA      420
TNTGAGCTAA TAGTAAAAAT GCGACTGTTT GCTTTTCAGA TTTTAACTT TGGCTAATAT      480
NTATGACTTN TTAAATGAA TGTTCGTGTA CTACATAATT CTATNTCAGA GACAGT      540

```

(1) INFORMATION FOR SEQ ID NO:16:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 650 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(II) MOLECULE TYPE: DNA (genomic)

(III) HYPOTHETICAL: NO

(IV) ANTI-SENSE: NO

(VII) IMMEDIATE SOURCE:

(B) CLONE: human alpha synuclein gene exon 4 plus
flanking intron sequences

(VIII) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4
(B) MAP POSITION: 4q21-q22



(x1) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```

CTGCAGGTCA ACGGATCTGT CTCTAGTGGT GTACTTTTAA AGCTTCTACA GTTCTGAATT      60
CAAAATTATC TTCTCACTGG GCGGCGGTGT TATCTCATTC TTTTTCCTCC TCTGTAAGTT      120
GACATGTGAT GTGGGAACAA AGGGGATAAA GTCATTATTT TGTGCTAAAA TCGTAATTGG      180
AGAGGACCTC CTCTTAGCTG GCGTTTCTTC TATNTATTGT GGTGCTTAGG AGTTCCTTCT      240
TCTAGTTTTA GGATATATAT ATATATTTTT TTCTTTCCTT GAGATATAA TAATATATAT      300
AGTTTCTTAC ATTGAGATTT TAAATTATG TATATTGAAA ACTTCTTAT TACCAATTTA      360
AGGCTAGCTT GAGACTTATG TCTTGAATTT GTTTTGTAG GCTCCAAAAA CAAGGAGGGA      420
GTGGTGCATG GTGTGGCAAC AGGTAAGCTC CATTGTGCTT ATATCAAGA TGATATNTAA      480
AGTATCTAGT GATTAGTGTG GCCCAGTATC AAGATTCTTA TGAAATTGTA AAACATTCAC      540
TGAGCATCTA AGAACATATC AGTCTTATTG AACTGAATT CTTTATAAAG TATTTTAAA      600
TAGGTAPATA TTGATTATTA ATAAAAATA TACTTGCCAA GAATAATGAG      660

```

(2) INFORMATION FOR SEQ ID NO:17:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 504 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

(111) HYPOTHETICAL: NO

(112) ANTI-SENSE: NO

(113) IMMEDIATE SOURCE:

- (B) CLONE: human alpha synuclein gene, exon 5 plus flanking intron sequences

(1111) POSITION IN GENOME:

- (A) CHROMOSOME/SEGMENT: 4
- (B) MAP POSITION: 4q21-q22

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```

ATATCTTAGC CAAGATTCAA TGTTGGTTG AACCACTC ACTTGACATC TTGGTGGCTT      60
TTGTTTCTTC TGACCACTCA GTTATCTATG GCATGTGTAG ATACAGGTGT ATGGAACGA      120
TGGCTASTGG AAGTGGAAATG ATTTTAAGTC ACTGTTATTC TACCACCCTT TAATCTGTTG      180
TTGCTCTTTA TTTGTACCAG TGGCTGAGAA GACCAAAGAG CAAGTGACAA ATGTTGGAGG      240

```



```

AGCAGTGGTG ACGGGTGTGA CAGCAGTAGC CCAGAAGACA GTGGAGGGAG CAGGGAGCAT      300
TGCAGCAGCC ACTGGGTTTT TCAAAAAGGA CCAGTTGGGC AAGGTATGGC TGTGTACGTT      360
TTGTGTTACA TTTATAAGCT GGTGAGATTA CGGTTTATTT TCATGTGAAG COTGGAGGCA      420
GGAGCAAGAT ACTTACTGTG GGAACGGCT ACCTGACCTT CCCCCTTGTGA AAAAGTGCTA      480
CCTTTATATT GGTCTTGCTT GTTT                                     504

```

2. INFORMATION FOR SEQ ID NO:18:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 527 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

(12) HYPOTHETICAL: NO

(13) ANTI-SENSE: NO

(14) IMMEDIATE SOURCE:

- (B) CLONE: human alpha synuclein gene exon 4 plus flanking intron sequences

(15) POSITION IN GENOME:

- (A) CHROMOSOME/SEGMENT: 4
- (B) MAP POSITION: 4q21-q22

3. SEQUENCE DESCRIPTION: SEQ ID NO:18:

```

AAAAGTTTAC ATACTTTGAG GTTGATAACC CATGTTGCCG CAATGTTTCC CCGGAGGCAT      61
TGTGGAGTTT AGAATGCCAG TAGTAATATT AAGGTGTGCC ATTTTGAAGA TGGTGGGCGA      121
ACATCCCTAT ATGTAAGATT TTTCCAAAAC ATGGTTCTGA TTTTAAAAAG TGAAAAATGC      181
TACTTCATCA TGTTCTTTTT GTGCTTCTTA CTTTAAATAT TAGAATGAAG AAGGAGGCCC      241
ACAGGAAGGA ATTGTGAAG ATATGCTGT GTATCCTGAC AATGAGGCTT ATGAATGCG      301
TTCTGAGGTA GGAATCCAAG CTGAATCTTT CTAACAAGAC AGTACAAAAA ACTTGTGATT      361
GTCACATTTG TCTTTCATTA GTGCTTAGTG AGAATCATTT GGTCTCTACA TGCTCATTAG      421
GTGGACAACT TGCAAGTTAA GAATAGTTTT TACATTTTTA AAGGGTCTCT AAAAAAAAAG      481
AGGAGGAGGA AGATGAAGAA GAGGAAGAAA GGATGTAAAA GAAATCATAT GTAGTCCACA      541
TAGCTTAATA TACNTACTAC TTGACCCCTT ACAGGAAAAG CTTTACTAAC CCCTGCATTA      601
GAGAATATAT TTTTGTGCAA AAACATTGAT TGTAATTTTT AGTGTAAAGT GCGGAGGCAT      661
TTGCTATCTG ATTGGGTTTT CAGTGCTGAT GCGTAATTGA AAGTTATATT AAGATGTTGT      721

```



GCTGTCT

727

[2] INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1596 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vii) IMMEDIATE SOURCE:
 (B) CLONE: human alpha synuclein gene exon 7 plus
 flanking intron sequences
- (viii) POSITION IN GENOME:
 (A) CHROMOSOME/SEGMENT: 4
 (B) MAP POSITION: 4q21-q22

[xi] SEQUENCE DESCRIPTION: SEQ ID NO:19:

```

TTTTGATTTT TCTAATATTA GGAAGGGTAT CAAGACTACG AACCTGAAGC CTAAGAAATA      60
TCTTTGCTCC CAGTTTCTTG AGATCTGCTG ACAGATGTTG CATCTGTAC AAGTGCTCAG      120
TTCCATATGT CCCAGTCATG ACATTTCTCA AAGTTTTTAC AGTGTATCTG GAAGTCTTCC      180
ATCAGCACTG ATTGAAGCAT CTGTACCTGC CCCCCTCAG CATTTCGGTG CTTCCTTTG      240
ACTGAAGTGA ATACATGGTA GCAGGGTCTT TGTGTGCTGT GGATTTTGTG GOTTCAATCT      300
ACGATGTAA AACAAATTAA AACACCTAA GTGACTACCA CTTATTTCTA AATCCTCACT      360
ATTTTTTTGT TGCTGTTGTT CAGAAATTGT TAGTGATTTG CTATCATATA TTATNAGATT      420
TTTAGGTGTC TTTTAATGAT ACTGTCTAAG AATAATGACG TATTGTGAAA TTTGTTAATA      480
TATATNATAC TTAAAAATAT GTGAGCATGA AACTATGCAC CTATAATACT AAATATGAAA      540
TTTTACCATT TTGCGATGTG TTTTATTCAC TTGTGTTTGT ATATNAATGG TGAGAATTAA      600
AATAAAACGT TATCTCATTG CAAAAATATT TTATTTTAT CCCATCTCAC TTATAATAA      660
AAATCATGTC TTATAAGCAA CATGAATTAA GAACTGACAC AAAGGACAAA AATATAAAGT      720
TATTAATAGC CATTGGAAGA AGGAGGAATT TTAGAAGAGG TAGAGAAAAT GGAACATTAA      780
CCCTACACTG GGAATTCCCT GAAGCAACAC TGCCAGAAGT GTGTTTTGGT ATGCACTGGT      840
TCCTTAAGTG GCTGTGATTA ATTATTGAAA GTGGGGTGTG GAAGACCCCA ACTACTATTG      900
TAGAGTGGTC TATTTCTCCC TTCAATCCTG TCAATGTTT CTTTACSTAT TTTGGGGAAC      960

```



TGTTGTTTGA	TGTGTATGTG	TTTATATTG	TTATACATTT	TTAATTGAGC	CTTTTATTAA	1020
CATATATTGT	TATTTTTGTG	TCGAAATAAT	TTTTTAGTTA	AAATCTATTT	TGTCTGATAT	1080
TGGTGTGAAT	GCTGTACCTT	TCTGACAATA	AATAATATNC	GACCATGAAT	AAAAAAAAAA	1140
AAAAAGTGGG	TTCCCGGGAA	CTAAGCAGTG	TAGAAGATGA	TTTTGACTAC	ACCCCTCCTTA	1200
GAGAGCCATA	AGACACATTA	GCACATATTA	GCACATTCAA	GGCTCTGAGA	GAATGTGCTT	1260
AACTTTGTTC	AACTCAGCAT	TCTCAGCTTT	TTTTTTTTTAA	TCATCAGAAA	TTCTCTCTCT	1320
CTCTCTCTTT	TTCTCTCTCT	CTCTTTTTTT	TTTTTTTTTT	TTTTACAGGA	AATGCCCTTA	1380
AACATCGTTG	GGAACTACCA	CAGTCACCTT	AAAGGGAGNA	TCAATTCTCT	AGGACTGGAT	1440
AAAAATTTCA	TGGGCTCTCT	TTAAATGTT	GGCCAAATAT	ATGGAAATCT	AGGGGTTTTT	1500
CCNTAGGGGG	AAGGGTTTTT	TCTCTTTTCN	GGGGAGGATC	CTTTTAAACN	CCCGGGGGGG	1560
NGCCCGGAAA	ATAAACTTGG	NGGGGGGGNA	AAACTT			1596



49. The method of claim 48 wherein said mutation is an alanine to threonine substitution

50. A diagnostic kit comprising the oligonucleotide of claim 41.

51. A diagnostic kit comprising the oligonucleotide of claim 42.

52. A diagnostic kit comprising the oligonucleotide of claim 43.

53. A diagnostic kit comprising the oligonucleotide of claim 7.

54. A diagnostic kit comprising the oligonucleotide of claim 8.

55. A diagnostic kit comprising the oligonucleotide of claim 9.

56. A diagnostic kit comprising the antibody of claim 23.

57. Canceled

58. Canceled

59. Canceled

60. Canceled



61. Canceled

62. A non-human transgenic animal which expresses a mutated synuclein protein, wherein said animal may be used as an animal model for Parkinson's disease.

63. The non-human transgenic animal of claim 62, wherein said mutated synuclein protein is an alpha synuclein protein.

64. A method of screening a compound for the ability to reverse the self-aggregation of synuclein proteins, comprising exposing an aggregate of synuclein proteins to a test compound and observing whether or not the aggregate is dissolved.

65. The method of claim 64 wherein said test compound is a synuclein peptide.

66. The method of claim 65 wherein said peptide comprises a mutation.

67. The method of claim 64 wherein said test compound is an antibody.

68. The method of claim 64, wherein said observing step comprises

AMENDED SHEET



49. The method of claim 48 wherein said mutation is an alanine to threonine substitution

50. A diagnostic kit comprising the oligonucleotide of claim 41.

51. A diagnostic kit comprising the oligonucleotide of claim 42.

52. A diagnostic kit comprising the oligonucleotide of claim 43.

53. A diagnostic kit comprising the oligonucleotide of claim 7.

54. A diagnostic kit comprising the oligonucleotide of claim 8.

55. A diagnostic kit comprising the oligonucleotide of claim 9.

56. A diagnostic kit comprising the antibody of claim 23.

57. Canceled

58. Canceled

59. Canceled

60. Canceled



61. Canceled

62. A non-human transgenic animal which expresses a mutated synuclein protein, wherein said animal may be used as an animal model for Parkinson's disease.

63. The non-human transgenic animal of claim 62, wherein said mutated synuclein protein is an alpha synuclein protein.

64. A method of screening a compound for the ability to reverse the self-aggregation of synuclein proteins, comprising exposing an aggregate of synuclein proteins to a test compound and observing whether or not the aggregate is dissolved.

65. The method of claim 64 wherein said test compound is a synuclein peptide.

66. The method of claim 65 wherein said peptide comprises a mutation.

67. The method of claim 64 wherein said test compound is an antibody.

68. The method of claim 64, wherein said observing step comprises

AMENDED SHEET



SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: The Government of the United States of America as represented by the Department of Health and Human Services at the National Institutes of Health
- (B) STREET: 6011 Executive Blvd., Suite 325
- (C) CITY: Rockville
- (D) STATE: Maryland
- (E) COUNTRY: USA
- (F) ZIP: 20852

(ii) TITLE OF INVENTION: Cloning of a gene mutation for Parkinson's disease

(iii) NUMBER OF SEQUENCES: 19

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(v) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: PCT/US98/13071
- (B) FILING DATE: 25-JUN-1998

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 216 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA genomic

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

- (B) CLONE: alpha synuclein gene/exon 4 region

(viii) POSITION IN GENOME:

- (A) CHROMOSOME/SEGMENT: 4
- (B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

GCTAATCAGC AATTAAAGG TAGCTTGAGA CTTATGTCTT GAATTTGTTT TTGTAGGCTC      60
CAAAACCAAG GAGGGASTGG TGCATGGTGT GACAACAGGT AAGCTCCATT GTGCTTATAT      120

```



CAAAGATGAT ATNTAAAGTA TCTAGTGATT AGTGFGGCCC AGTATCAAGA TTCTATGAA 180
ATTGTAAAAC AATCACTGAG CATCTAAGAA CATATC 216

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: desc = "primer #3"

(iii) HYPOTHETICAL: NO

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GCTAATCAGC AATTTAGGCT AG

22

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: desc = "primer #13"

(iii) HYPOTHETICAL: NO

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTATACAAGA ATCTACAGT C

21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 140 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (C) INDIVIDUAL ISOLATE: Swiss-Prot P37840



(vii) IMMEDIATE SOURCE:
 (B) CLONE: alpha synuclein protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
1           5           10           15
Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
20           25           30
Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
35           40           45
Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
50           55           60
Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
65           70           75           80
Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys
85           90           95
Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile
100          105          110
Leu Glu Asp Met Pro Val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro
115          120          125
Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
130          135          140

```

(xii) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 140 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(iii) MOLECULE TYPE: peptide

(iiii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) ORIGINAL SOURCE:
 (A) ORGANISM: Rattus norvegicus
 (C) INDIVIDUAL ISOLATE: Swiss-Prot P37377

(viii) IMMEDIATE SOURCE:
 (B) CLONE: alpha synuclein protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
1           5           10           15

```



Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
 20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45

Val His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
 50 55 60

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
 65 70 75 80

Thr Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Phe Val Lys
 85 90 95

Lys Asp Gln Met Gly Lys Gly Glu Glu Gly Tyr Pro Gln Glu Gly Ile
 100 105 110

Leu Glu Asp Met Pro Val Asp Pro Ser Ser Glu Ala Tyr Glu Met Pro
 115 120 125

Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
 130 135 140

(i) INFORMATION FOR SEQ ID NO:6:

(ii) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 134 amino acids
- (B) TYPE: amino acid
- (C) TOPOLOGY: linear

(iii) MOLECULE TYPE: peptide

(iv) HYPOTHETICAL: NO

(v) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Bos taurus
- (C) INDIVIDUAL ISOLATE: Swiss-Prot P33867

(vii) IMMEDIATE SOURCE:

- (B) CLONE: alpha synuclein protein

(viii) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asp Val Phe Met Lys Gly Leu Ser Met Ala Lys Glu Gly Val Val
 1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Thr Glu Ala Ala Glu Lys
 20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45

Val Gln Gly Val Ala Ser Val Ala Glu Lys Thr Lys Glu Gln Ala Ser
 50 55 60



His Leu Gly Gly Ala Val Phe Ser Gly Ala Gly Asn Ile Ala Ala Ala
 65 70 75 80
 Thr Gly Leu Val Lys Lys Glu Glu Phe Pro Thr Asp Leu Lys Pro Glu
 85 90 95
 Glu Val Ala Gln Glu Ala Ala Glu Glu Pro Leu Ile Glu Pro Leu Met
 100 105 110
 Glu Pro Glu Gly Glu Ser Tyr Glu Glu Gln Pro Gln Glu Glu Tyr Gln
 115 120 125
 Glu Tyr Glu Pro Glu Ala
 130

2. INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 142 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: peptide

 (iii) HYPOTHETICAL: NO

 (iv) ANTI-SENSE: NO

 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Serinus canaria*
 (C) INDIVIDUAL ISOLATE: genbank L33860

 (vii) IMMEDIATE SOURCE:
 (B) CLONE: alpha synuclein homologue

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Val Val Ala
 1 5 10 15
 Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys Thr
 20 25 30
 Lys Glu Gly Val Leu Tyr Val Gly Ser Arg Thr Lys Glu Gly Val Val
 35 40 45
 His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Ser Asn
 50 55 60
 Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys Thr
 65 70 75 80
 Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Leu Val Lys Lys
 85 90 95
 Asp Gln Leu Ala Lys Gln Asn Glu Glu Gly Phe Leu Gln Glu Gly Met
 100 105 110



Val Asn Asn Thr Gly Ala Ala Val Asp Pro Asp Asn Glu Ala Tyr Glu
 115 120 125

Met Pro Pro Glu Glu Glu Tyr Gln Asp Tyr Glu Pro Glu Ala
 130 135 140

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 143 amino acids
 (B) TYPE: amino acid
 (C) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Torpedo californica
 (C) INDIVIDUAL ISOLATE: Swiss-Prot P37379
- (vii) IMMEDIATE SOURCE:
 (B) CLONE: alpha synuclein homologue

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Asp Val Leu Lys Lys Gly Phe Ser Phe Ala Lys Glu Gly Val Val
 1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Gln Asp Ala Ala Glu Lys
 20 25 30

Thr Lys Gln Gly Val Gln Asp Ala Ala Glu Lys Thr Lys Glu Gly Val
 35 40 45

Met Tyr Val Gly Thr Lys Thr Lys Glu Gly Val Val Gln Ser Val Asn
 50 55 60

Thr Val Thr Glu Lys Thr Lys Glu Gln Ala Asn Val Val Gly Gly Ala
 65 70 75 80

Val Val Ala Gly Val Asn Thr Val Ala Ser Lys Thr Val Glu Gly Val
 85 90 95

Glu Asn Val Ala Ala Ala Ser Gly Val Val Lys Leu Asp Glu His Gly
 100 105 110

Arg Glu Ile Pro Ala Glu Gln Val Ala Glu Gly Lys Gln Thr Thr Gln
 115 120 125

Glu Pro Leu Val Glu Ala Thr Glu Ala Thr Glu Glu Thr Gly Lys
 130 135 140

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:



- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer #1F"

- (iii) HYPOTHETICAL: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACGACAGTGT GTGTAAAGG

19

- (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer #13R"

- (iii) HYPOTHETICAL: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AACATCTGTC AGCAGATCTC

20

- (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2804 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: BAC clone 139A20 Human Beta Synuclein Gene

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCGCCGCAGC CGCCGCTCCA TCCCAGCCC CGGCCCGCA TCCGGTTTGG AAGGGGGCTG

60



CAAGTTTGCA AGGGGCCCCG GANAAAAANC GAGCAGTGGC CCTTCCCCGC TCCCCAGGGT	120
TTCAAGGGAC GCTAGGANTN TCCGCGGCCC TGGAGGTTG CACTGGGGAG TGGGGTGAGA	180
TGGGGGAAA GCGGGAGGGG GTCAGGGTC CAGAAGGGCN CCGCGGTGT GGGASTAGGG	240
GGGCATNTGC GTCCCGGGG AGGGGCTGGG GTGAGAGTGC GGGGCCAGTG CACGGGTGCG	300
CGTSTATCGC CCTCCCCAGG CCGCCAGGAT GGACGTGTTT ATGAAGGGCG TGTCCATGGC	360
CAAGGAGGGG GTTGTGGGAG CCGCGAGAAA AACCAAGCAG GGGGTCACTG AGGCGGGGGA	420
GAAGACCAAG GAGGGCGTCT TCTACGTGG TGGGCGGGG GCGGGGTTTT TGGGGGTGCA	480
GGGTGGGGG TCCCCCTATA GTTGTGAGGT GCGCGCGGCT CCGGTGGAGG GGGGTCTCTC	540
GCAAGATAAT ATNANTCAGC AGATGGGGCN AGGTGANCAN GGGTCTATAA GGACATACCC	600
ANCCCATAGA ANCCCTGGTC TGTATCTGGA AATGGGGACA CCGGCGGGGC TGATGAGGTG	660
GGGGGGCTCA NCTGAAAGGC CAGGACCCAN TGCANTNATA AAANACACA NCCTCCTTTT	720
TCTATCTTTT TTTACCATTA TTAATAGTTA TCTGGTGTG AACATTTTCT GTATGCAAG	780
TACTGGGTAA AATGTCATAA CATCCATTTT CTCATGTAAT GGTTCGGGCG ATTCTAAGG	840
TAAAGGAAAC TGGGCTTCCC ATTGGTAGNT AAATTTTAGG TTCAGAAAGG CTGGAATTGA	900
ATGTCAGTTC AGCCAAATTC TTAGTGGTGG AACCAAACTG AGTTCCATCC GTGAAATGGG	960
GACAATAACA GCACCCGCTT CCCAGGGCTG GGGAAAAGTG AAGTGCAGCG GGGCAGGCAG	1020
AGGACTTGAC ACAGCACTGG CCCTCAGCCA ACATCCACTA GAGGGGTGGG GTATCGCATC	1080
AGGTGGGAGA GAATGCAAC CCTTGCAGAC AGAGGTGTGG GGGCCAGTGC AGTGATAAGA	1140
CGGGGCTTAA CATGGGGTG CAGTTGTAG GATNTGGGGA CCGAAGGAGG CATTGACGGG	1200
GCCAGGATGC CCACTGTGTA ATCACCATGC TGTGCTGGAG TTTCTGTTTC CTCAGCGGAG	1260
AGTCCTTAAA TGTCCCGCTT TTTCTNCCCT GAGGAAGCA AGACCCGAGA AGTGTGTGTA	1320
CAAGGTGTGG CTTCAGGTAC TAGCCGAGCC CTGGCAGCAG CCCTTCTCTC AMTTAGGGGG	1380
ATGATCTGGC CGGGAACGAG AGGGCGGGGG GGGGGAGAC TCCCAAGGCT TCTGCGGGAA	1440
TGCTCGGTGG GGAGGGCAGG CCCTGGGATA CTAAAGGCA GGGCATCGGT GTTTCCCGCT	1500
GGTTCGAAA CCGCTTCTTC AACCTCTCTC CTCTCTCAGT GGCTGAAAA ACCAAGGAAC	1560
AGGCTCACA TCTGGGAGGA GGTGTCTCT CTGGGGCAGG GAACATCGCA GCAGCTACAG	1620
GACTGGTGAA GAGGGAGGAA TTCCCTACTG ATCTGAAGGT AAGCGATCCT TCTGACCGGC	1680
ACATGCAGGC AAACACACAC ACACACACAC ACACACACCN GGCACACAAA TAAACCTGTC	1740
ACCATCCCGG CCCCCCTAAT CCTGCCACCA GCTTGGAAAC CAAGCCACTT TGCCTCCCAT	1800
CCTGCGGGCC CGTGCTAGAC TCAGCTCAGA ATGCATCTGA ATAANGGCT GCATGGGTET	1860



GACGCTCCCG GTGATGGGGA CCCAGACCTG GCTGTCTGCG TGTATCCTGC TTGCCAGCGT 1920
 GACCCATATG ACTTCTGGCC ACGTCTGCAT GTGTCAATGA TTGTTTCATTC ATTTCTTTTC 1980
 ATTCAACAAA TATCCATGCC ANANCCAGCC CTGTCTTGA GCTTCCAGNT CCCTTTCAGC 2040
 CNAGGGGAGC NTGAGGGTTA TTTTGGGGT CCGGATGCCC AGCACAGAGC CTGACACAAA 2100
 GGATGAGGCA TAAGCTGGTG ANTGAGTATC CAAATGGTGG AAGTGTGCAG GNTGCCAGGC 2160
 ATTGGGGGAG CGGCGTGGAG AGCCAGCTCC CCAATCCATG CTGCCACTTC AACTGTGATT 2220
 CGGGGGAATT TCCCCCTTCA CCTCCATCCC ACTTCCAAGG CACTCCAAAT AAATAACTGA 2280
 ATTAGAAATT ATCCTTGTTC TCCCAACCA CCTAGCCTT CCCCCTCCA ACCCAACCA 2340
 AGCTTACCAC TGTGGGAATT TGGGGGGCAT CCTGGCTGTC CTCACGAGTC CTGACCTTTT 2400
 CTGCCCCACAG CCAGAGGAAG TGGCCAGGA AGCTGCTGAA GAACCACTGA TTGAGCCCTT 2460
 GATGGAGCCA GAAGGGGAGA GTTATGAGGA CCCACCCCAG GAGGAATATC AGSAGTATGA 2520
 GCCAGAGGCG TAGGGGCCCA GGAGAGCCCC CACCAGCAGC ACAATTCTGT CCTGTCCCT 2580
 GCGCGCCCCC CCAGAGCCAG GGCTGTCTT AGACTCTTC TCCCCAATCA CGAGATCTTC 2640
 CTCGCTCTT GAGGCAACCC CCTCGGAGCC TGTGTTAGTG TCTGTGCATC TGTCTGTCT 2700
 ACCCGCCCCG GTCCAACCCC GGGGCATGGA CAGGGCCAGG GTTGCGGTG CGGCTGGGAG 2760
 CCTCGCCCCT CCAGTGTGTC CTCCTCCCAT CCAGCGTCTG CGCG 2804

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 223 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

- (B) CLONE: BAC clone 174P13 Human Gamma Synuclein Gene,
5' end

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGGGAGATCC AGCTCCGTCC TGCCTGCAGC AGCACAACCC TGCACACCCA CCATGGATGT 60
 CTTCAAGAAG GGCTTCTCCA TCGCCAAGGA GGGNGTGGTG GGTGCGGTGG AAAAGACCAA 120
 GCAGGGGGTG ACGGAAGCAG CTGAGAAGAC CAAGGAGGGG GTCATGTATG TGGGATTACA 180



TTTTTTTTTTT AAAGAAAGAA TAAATTAATT GTGATTAAAG TTG

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 677 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: BAC clone 174P13 Human Gamma Synuclein Gene,
3' end

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTTTTTNAGG GGGGAAAACA GSGAATANAA AAANANGGGG GGGGGTTTTT NNGGGGGGGG	60
GGGGAAAANG GTTNGGGGGG NAACCNAAAN AAANNCCNAN GGGGGGGGNN ANTNAANTTT	120
TGGGAACCCA AAGCCCNAGG AGGATTTTTT GTNAANAACG TNACCTCNAG TGGGNCGAGG	180
AAGACCAAGG AAANGCCCAA CNGGTTGAN CGAGGCTGTG GTGAACANCG TNCAACNCTG	240
TGCCCCCCAA NANGTGGAG GNGGCGGAGA ACATCSGGT CACCTCCGGG GTGGTGCGCM	300
AGGAGGACTT GAGGCCATCT TCCCCCMAA AGGAGGGTGT GGCATCCMAA GARAAAGAGG	360
AAGTGGCAGA GGAGGCCAG AGTGGGGGAR ACTAGAGGGT TACAGGCCAG CGTGGATGAC	420
CTGAAGAGCG CTCCTCTGCC TTGGACACCA TCCCCTCCTA GCACAAGGAG TGCCCGCCTT	480
GAGTGACATG CGGCTGCCCC CGCTCCTGCC CTCGTCTTCC TGGCCACCCT TGGCCTGTCC	540
ACCTGTGCTG CTGCACCAAC CTCACTGCCC TCCCTCGGCC CCACCCACCC TCTGGTCTCT	600
CTGACCCAC TTATGCTGCT GTGAATTTTT TTTTAAATG ATTCCAAATA AACTTGAGC	660
CCACTCCAAA AAAAAA	677

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1181 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO



(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(B) CLONE: human alpha synuclein gene exons 1 and 2 plus flanking intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```

AAATTCACTG ATGCGAGGGG AAAAGCGCTGT TTTTCTTGGG GTGTGAGGGA CTTTCTTGGG
CTGCTGTGTCT CCTCCAGCAG CTCCCGAAGG GATAGGCTGT GCGCTTGGTG GTCGAGCGTC
AGGCGCTCGN TCTCCAGGN CGACTCTGAC GAGGCGTAGG GGGTGGTCCC CNGGAGGACC
CAGAGGAAG GGNBGCACAA GAAGGAGGG GAAGGGGAAA GAGGAAGAGG CATCATCCCT
AGCGCAAGCG CTCCCGATCT CCAAGAAGAGT GCTGCTGACC CTAAACTTAA CGTGAGGCGC
AAAAGCGCGG CAACCTTTTC CCGCTTGNV CGAGCGAGGG GGCTGAGATT GATGCTGAC
CGCGCGCGCG CTGCGCGCATC CCGATCTGAG ATAGGACGGA GGAGCAGCT GCAGGGGAG
CAGCGAGCGG CCGGAGAGGG GCGCGCAGAA GCGCTGACAA ATCAGCGGTG GGGGCGGAGA
GCTGAGGAGA AGGAGAAGGA GAGGAGTAG GAGGAGGAG ACGGCGAGGA CCAGAAGGGG
CCGAAGAGAG GCGCGCAGCG ACCGAGCGCG GCGACCGGAA GTGAGCTCG TCGGGGCTCA
GCGCAGACCT CCGCGCGGGG CTTCTTGA GAAGCTGCGG CGCTCCCTCA CGCTTGGCT
TGAAGCGCTT TCGCTTTTCA CCGCTGAG CCGAGAACTG GGAGTGGCCA TTGAGAGACA
GGTAGCGGG TTTGCGTCCC ACTCGCGCAG CCGCGCTCG CCGGCTCACA GCGGCTGCT
CTGGGGACAG TCGCGCGCGG GTGCGCTTC GCGCTTCTG TCGGCTGCTT TCGCTTCTC
TTTCTATTA AATATTATTT GCGAATTGT TAAATTTTTT TTTTAAAAA AGAGAGAGGC
GNGGAGGAGT CGGAGTTGT GAGAAAGAGA GGGACTCAGG TAAGTACCTG TGGATCTAAA
CGGNGTCTT TGGAAATCT GAGAAAGGCG GGATGGAGAG GAATGGTCTT GCGNACCGGG
AGGCGGTGGT GCTGCGATGA GAGCGCTGG GCCAGGTCTC TGGGAGGTGA GTACTTGTCC
TTTGGGGAGC CTAAGCAAG AGACTTGACC TGGCTTCTG CCGCTTCTG ATATTGCTT
CTCCACAAGG GCTGAGAGNT TAGGCTGCTT CTGGGGGATC C

```

(xii) INFORMATION FOR SEQ ID NO:15:

1. SEQUENCE CHARACTERISTICS:

(A) LENGTH: 536 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double



(D) TOPOLOGY: linear

(iii) MOLECULE TYPE: DNA (genomic)

(iiii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(B) CLONE: human alpha synuclein gene exon 3 plus
flanking intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4
(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```

CTTAAAGAG TCTCAGACTT TGGAGGGTTT CTCATGATTT TTCAGTGTTC TTTGTTTATT      60
TTTCCCCGAA AGTTCTCATT CAAAGTGTAT TTTATGTTTT CCAGTGTGGT GTATAGAAAT      120
TCATTAGCCA TGGATGTATT CATGAAAGGA GTTTCAAAGG CCAAGGAGGG AGTTGTGGCT      180
GCTGCTGAGA AAACCAACA GGGTGTGGCA GAAGCAGCAG CAAAGACAAA AGAGGCTGTT      240
CTCATGTAG STAGGTAAAC TCCAAATGTC AGTTTGGTGC TTGTCATGA STGATGGGTT      300
AGGATACAAA TACTCTAAAT GCTGGTAGTT CTCTCTCTTG ATTCATTTTT GCATCATTGC      360
TTGTCAAAAA GGTGGACTGA CTCAGAGGTA TGTGTAGGTA GGTGAATGTG AACGTGTGTA      420
TNTGAGCTAA TAGTAAAAAT GCGACTGTTT GCTTTTCAGA TTTTAAATTT TGCTAATAT      480
NTATGACTTN TTAAATGAA TGTTCGTGTA CTACATAATT CTATNTCAGA GACAGT      536

```

(x) INFORMATION FOR SEQ ID NO:16:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 650 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(iii) MOLECULE TYPE: DNA (genomic)

(iiii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(B) CLONE: human alpha synuclein gene/exon 4 plus
flanking intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4
(B) MAP POSITION: 4q21-q22



(x1) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```

CTGCAGGTCA ACGGATCTGT CTCTAGTGCT GTACTTTTAA AGCTTCTACA GTTCTGAATT      60
CAAAATTATC TTCTCACTGG GCGCCGGTGT TATCTCATTC TTTTCTCTCC TCTGTAAGTT      120
GACATGTGAT GTGGGAACAA AGGGGATAAA GTCATTATTT TGTGCTAAAA TCGTAATTGG      180
AGAGGACCTC CTGTTAGCTG GGCTTTCTTC TATNTATTGT GGTGGTTAGG AGTTCCTTCT      240
TCTAGTTTTA GCATATATAT ATATATTTTT TCTTTTCCCT GAAGATATAA TAATATATAT      300
ACTTCTGTAAG ATTGAGATTT TTAATTAGT TATATTGAAA ACTATTTAAT GAGCAATTTA      360
AGGCTAGCTT GAGACTTATG TCTTGAATTT GTTTTGTAG GCTCCAAAAC CAAGGAGGGA      420
GTGGTGCAAG GTGTGGCAAG AGGTAAGCTC CATTGTGCTT ATATCAAAGA TGATATNTAA      480
AGTATCTAGT GATTAGTGTG GCCCAGTATC AAGATTCCCTA TGAAATTGTA AAACAATCAC      540
TGAGCATCTA AGAACATATC AGTCTTATTG AAAGTGAATT CTTTATAAAG TATTTTTTAA      600
TAGGTAAATA TTGATTATAA ATAAAAAATA TACTTGCCAA GAATAATGAG      660

```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 504 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(viii) IMMEDIATE SOURCE:

- (B) CLONE: human alpha synuclein gene/exon 5 plus flanking intron sequences

(viii) POSITION IN GENOME:

- (A) CHROMOSOME/SEGMENT: 4
- (B) MAP POSITION: 4q21-q22

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```

ATATCTTAGC CAAGATTCAA TGTTTGGTTG AACCACACTC ACTTGACATC TTGGTGGCTT      60
TTGTTTCTTC TGACCACTCA GTTATCTATG GCATGTGTAG ATACAGGTGT ATGGAANCGA      120
TGGCTASTGG AAGTGGAAATG ATTTTAAGTC ACTGTTATTC TACCACCCCTT TAATCTGTTG      180
TTGCTCTTTA TTTGTACCAG TGGCTGAGAA GACCAAAGAG CAAGTGACAA ATGTTGGAGG      240

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AGCAGTGGTG ACGGGTGTGA CAGCAGTAGC CCAGAAGACA GTGGAGGGAG CAGGGAGCAT      300
TGCAGCAGCC ACTGGGCTTTG TCAAAAAGGA CCAGTTGGGC AAGGTATGGC TGTGTACGTT      360
TTGTGTTACA TTTATAAGCT GGTGAGATTA CGGTTTCATTT TCATGTGAAG COTGGAGGCA      420
GGAGCAAGAT ACTTACTGTG GGGAAAGGCT ACCTGACCCT CCCCTTGTGA AAAAGTGCCTA      480
CCTTTATATT GGTCTTGCTT GTTT                                     504

```

2. INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 707 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: human alpha synuclein gene/exon 6 plus flanking intron sequences
- (viii) POSITION IN GENOME:
 - (A) CHROMOSOME/SEGMENT: 4
 - (B) MAP POSITION: 4qQ1-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```

AAAAGTTTAC ATACTTTGAG GTTGATAAGC CATGTTGCGG CAATGTTTCC CCGGAGGCAT      60
TGTGGAGTTT AGAATGCCAG TASTAATATT AAGGTGTGCC ATTTTCAAGA TCGGTGGCCA      120
ACATCCCATAT ATGTAAGATT TTTCCAAAAC ATGGTTCTGA TTTTAAAAAG TGAAAAATGC      180
TACTTCATCA TTTCTTTTTT GTGTTCTTCA CTTTAAATAT TAGAATGAAG AAGGAGCCCC      240
ACAGGAAGGA ATTCTGGAAG ATATGCTGTG GGATCCTGAC AATGAGGCTT ATAAATGCG      300
TTCTGAGGTA GGAGTCCAAG CTGAATCTTT CTAACAAGAC AGTACCAAAA ACCTGTCATT      360
GTCACATTTT TCTTTCATTA GTGCTTAGTG AGAATCATTT GCTCTCTACA TGCTCATTAC      420
GTGGACAACCT TGCAAGTTAA GAATAGTTTT TACATTTTTTA AAGGTCCTT AAAAAAAAAAG      480
AGGAGGAGGA AGATGAAGAA GAGGAAGAAA GGATGTAAAA GAAATCATAT GTAGTCCACA      540
TAGCTTAATA TACNTACTAC TTGACCCTTT ACAGGAAAAG CTTTACTAAC CCTTGCATTA      600
GAGAATATAT TTTTTTGCAA AAACATTGAT TGTAAATTTT AGTGTAAGT GGGGAGGCCAT      660
TTCTATCTG ATTGGATGTG CAGTGTGAT GCGTAATTGA AACTTATAGT AATAATGTGT      720

```



GCTGTCT

707

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1596 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

- (B) CLONE: human alpha synuclein gene/exon 7 plus flanking intron sequences

(viii) POSITION IN GENOME:

- (A) CHROMOSOME/SEGMENT: 4
- (B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```

TTTTGATTTT TCTAATATTA GGAAGGGTAT CAAGACTACG AACCTGAAGC CTAAGAAATA      60
TCTTTGCTCC CAGTTTCTTG AGATCTGCTG ACAGATGTTT CATCCTGTAC AAGTGCTCAG      120
TTCCAAATGTG CCCAGTCATG ACATTTCTCA AAGTTTTTAC AGTGTATCTC GAAGTCTTCC      180
ATCAGCAGTG ATTGAAGCAT CTCTACCTGC CCCCACTCAG CATTTCGGTG CTTCCCTTTC      240
ACTGAAGTGA ATACATGGTA GCAGGGTCTT TGTGTGCTGT GGATTTTGTG GCTTCAATCT      300
ACGATGTTAA AACAAATTAA AAACACCTAA GTGACTACCA CTTATTTCTA AATCCTCACT      360
ATTTTTTTGT TGCTGTTGTT CAGAACTTGT TAGTGATTTT CTATCATATA TTATNAGATT      420
TTTAGGTGTC TTTTAATGAT ACTGTCTAAG AATAATGACG TATTGTGAAA TTTGTTAATA      480
TATATNATAC TTAAAAATAT GTGAGCATGA AACTATGCAC CTATAATACT AAATATGAAA      540
TTTTACCAAT TTGGGATGTG TTTTATTCAC TTGTGTTTGT ATATNAATGG TGAGAATTAA      600
AATAAAACGT TATCTCATTG CAAAAATATT TTATTTTTAT CCCATCTCAC TTTAATAATA      660
AAAATCATGC TTATAAGCAA CATGAATTAA GAACTGACAC AAAGGACAAA AATATAAAGT      720
TATTAATAGC CATTGGAAGA AGGAGGAATT TTAGAAGAGG TAGAGAAAAT GGAACATTAA      780
CCCTACACTC GGAATTCCTT GAAGCAACAC TGCCAGAAGT GTGTTTTGGT ATGCACTGGT      840
TCCTTAAGTG GCTGTGATTA ATTATTGAAA GTGGGCTGTT GAAGACCCCA ACTACTATTG      900
TAGAGTGSTC TATTTCTCCG TTCAATCTTG TCAATGTTTG CTTTACGTAT TTTGGGGAAC      960

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TGTTGTTTGA	TGTGTATGTG	TTTATAATTG	TTATACATTT	TTAATTGAGC	CTTTTATTAA	1020
CATATATTGT	TATTTTTGTC	TCGAAATAAT	TTTTTAGTTA	AAATCTATTT	TGTCTGATAT	1080
TGGTGTGAAT	GCTGTACCTT	TGTGACAATA	AATAATATNC	GACCATGAAT	AAAAAAAAAA	1140
AAAAAGTGGG	TTCCCGGGAA	CTAGGCAGTG	TAGAAGATGA	TTTTGACTAC	ACCCTCCTTA	1200
GAGAGCCATA	AGACACATTA	GCACATATTA	GCACATTCAA	GGCTGTGAGA	GAATGTGGTT	1260
AACTTTSTTT	AACTCAGCAT	TCTCAGCTTT	TTTTTTTTTAA	TCATCAGAAA	TTCTCTCTCT	1320
CTCTCTCTTT	TTCTCTCGCT	CTCTTTTTTT	TTTTTTTTTT	TTTTACAGGA	AATGCCTTTA	1380
AACATCGTTG	GGAACTAGCA	GACTCAGCTT	AAAGGGAGNA	TCAATTCTCT	AGGACTGGAT	1440
AAAAATTTCA	TGGGCTCTCT	TTAAAATGTT	GCCCAAATAT	ATGGAATTCT	AGGGGTTTTT	1500
CCNTAGGGGG	AAGGGTTTTT	TCTCTTTTCT	GGGAGGATC	CTTTTAACNC	CCCGGGGGGG	1560
NGCCCCGAAA	ATAAACTTGG	NGGGGGGGNA	AAACTT			1596

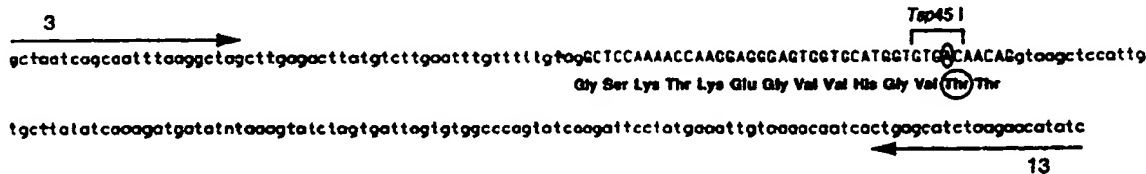




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/12, C07K 14/47, C12N 15/11, C07K 16/18, A61K 48/00, C12Q 1/68, G01N 33/68, A01K 67/027		A1	(11) International Publication Number: WO 98/59050 (43) International Publication Date: 30 December 1998 (30.12.98)
(21) International Application Number: PCT/US98/13071 (22) International Filing Date: 25 June 1998 (25.06.98) (30) Priority Data: 60/050,684 25 June 1997 (25.06.97) US		201, 14020 Old Harbor Lane, Marina Del Ray, CA 90292 (US). (74) Agents: SCHNELLER, John, W. et al.; Spencer & Frank, Suite 300 East, 1100 New York Avenue, N.W., Washington, DC 20005-3955 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Office of Technology Transfer, National Institute of Health, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): POLYMEROPOULOS, Mihael, H. [US/US]; 8301 Raymond Lane, Potomac, MD 20854 (US). LAVEDAN, Christian [FR/US]; 14421 Frances Green Way, North Potomac, MD 20878 (US). LEROY, Elisabeth [FR/US]; 4316 Garrison Street, N.W., Washington, DC 20016 (US). NUSSBAUM, Robert, L. [US/US]; 3815 Leland Street, Chevy Chase, MD 20815 (US). JOHNSON, William, G. [US/US]; 91 Stewart Road, Short Hills, NJ 07078 (US). DUVOISIN, Roger, C. [US/US]; Apartment		Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	

(54) Title: CLONING OF A GENE MUTATION FOR PARKINSON'S DISEASE



(57) Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder with a lifetime incidence of approximately 2 percent. It was recently reported that a PD susceptibility gene is located on the long arm of human chromosome four. The present invention reports the subsequent identification of a mutation in the alpha synuclein gene, which codes for a presynaptic protein thought to be involved in neuronal plasticity. The finding of a specific molecular alteration which is causative for PD will permit the detailed understanding of the pathophysiology of the disorder, which will lead to potential therapeutic interventions, as well as a means for diagnosing individuals having an increased risk of developing the disease.



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CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						



CLONING OF A GENE MUTATION FOR PARKINSON'S DISEASE

5 BACKGROUND OF THE INVENTION

1. Field of the Invention

Parkinson's disease (PD) is a common neurodegenerative disorder with a lifetime incidence of approximately 2 percent. A pattern of familial aggregation has been documented for the disorder, and it was recently reported that a PD susceptibility gene in a large Italian kindred is located on the long arm of human chromosome 4. We have identified a mutation in the alpha synuclein gene, which codes for a presynaptic protein thought to be involved in neuronal plasticity, in the Italian kindred and in three unrelated families of Greek origin with autosomal dominant inheritance for the PD phenotype. This finding of a specific molecular alteration which is causative for PD will permit the detailed understanding of the pathophysiology of the disorder. In addition, methods of screening nucleic acids for the presence of mutations in the synuclein gene to test for predisposition to Parkinson's Disease are now possible.

25 2. Technology Background

Parkinson's disease (PD) was first described by James Parkinson in 1817 (1). The clinical manifestations of this



neurodegenerative disorder include resting tremor, muscular rigidity, bradykinesia and postural instability. A relatively specific pathological feature accompanying the neuronal degeneration is the intracytoplasmic inclusion body, known as the Lewy body, which is found in many regions including the substantia nigra, locus ceruleus, nucleus basalis, hypothalamus, cerebral cortex, cranial nerve motor nuclei, and the central and peripheral divisions of the autonomic nervous system (1).

In many cases a heritable factor predisposes to the development of the clinical syndrome (2). We have recently shown that genetic markers on human chromosome 4q21-q23 segregate with the PD phenotype in a large family of Italian descent (3). The clinical picture of the PD phenotype in the Italian kindred has been well documented to be typical for PD, including Lewy bodies, with the exception of a relatively earlier age of onset of illness at 46 ± 13 years. In this family the penetrance of the gene has been estimated to be 85%, suggesting that a single gene defect is sufficient to determine the PD phenotype.

We now report the identification of a mutation in the alpha synuclein gene that is associated with Parkinson's disease. The mutation, an Ala53Thr substitution, was found to be linked to the PD phenotype in four independent PD families and absent from 314 control chromosomes, providing strong genetic evidence that this mutation in the human alpha



synuclein gene is causative for the PD phenotype in these families.

The Ala53Thr substitution is localized in a region of the protein whose secondary structure predicts an alpha helical formation, bounded by beta sheets. Substitution of the alanine with threonine is predicted to disrupt the alpha helix and extend the beta sheet structure. Beta pleated sheets are thought to be involved in the self aggregation of proteins which could lead to the formation of amyloid like structures (6).

This was already tested in the case of NAC35, the 35 amino acid peptide derived from alpha-synuclein that was first isolated from plaques found in patients with Alzheimer's disease (4). NAC35 was shown to self aggregate and form amyloid fibril which shared the 'amyloid' characteristics of insolubility in aqueous solutions and green birefringence under polarized light, subsequent to Congo red staining (6). NAC35 is located in the middle of the alpha synuclein molecule and extends from amino acid 61 to amino acid 95. Residue 53, which is found to be mutated in PD, is outside the NAC35 peptide found in amyloid plaques. However, the true size of the NAC peptide involved in the plaques is not known since the protease used to isolate the peptide from AD tissue cuts at lysine 60 of the alpha synuclein protein. It is therefore possible that amino acid 53 may be part of the NAC peptide found in plaques. In



crosslinking experiments with beta amyloid (Abeta), it was demonstrated (6) that residues 1-56 and 57-97 specifically bind amyloid and that a synthetic peptide consisting of residues 32-57 performed similarly.

5 Three members of the synuclein family have been characterized in the rat, with SYN1 exhibiting 95% homology with the human alpha-synuclein protein (7). SYN 1 of the rat is expressed in many regions of the brain, with high levels found in the olfactory bulb and tract, the hippocampus,
10 dentate gyrus, habenula, amygdala and piriform cortex, and with intermediate levels in the granular layer of the cerebellum, substantia nigra, caudate-putamen, and dorsal raphe (7). This pattern of expression coincides with the distribution of the Lewy bodies found in brains of patients
15 with Parkinson's disease. It is also interesting to note that decrease in olfactory sense often accompanies the syndromic features of Parkinson's disease, and in many cases it is proposed that hyposmia is a prodromic sign of the illness (8).

20 In the zebra-finch the homologue to alpha synuclein, synelfin, is thought to be involved in the process of song learning, suggesting a role for synuclein perhaps in memory and learning (9). In contrast to humans, rats have a threonine at residue 53 of their homologues to the human
25 alpha synuclein gene (Figure 4). Similarly, the zebra-finch synelfin carries a threonine at amino acid 53, whereas both



Bos taurus and Torpedo californica do not (10). There are no reports that suggest the presence of Lewy bodies in the brains of the rat or the zebra finch or a phenotype resembling that of PD. Lack of any phenotype could be explained by a combination of factors, including the following: the relative short life span of rodents may prohibit the observation of a late onset disorder, interaction with other cellular components not present in the rat may be required for the phenotype, absence of a critical environmental trigger in the rodents, or finally a heterozygous status Ala/Thr may be necessary for the production of a phenotype.

Studies of early onset AD have previously documented that missense mutations can cause an adult onset neurodegenerative disorder. Of the 31 mutations described so far in the loci for presenilin 1 and 2, thirty were missense and one was a splice variant (11). Missense mutations in the prion protein have also been implicated in the amyloid production seen in Gerstmann-Straussler-Scheinker and Creutzfeldt-Jakob diseases, both forms of spongiform encephalopathy (12). Studies in these neurodegenerative disorders have pointed to the importance of the physical chemical properties of mutant cellular proteins in initiating and propagating neuronal lesions leading to disease. Similar studies in the synuclein protein family may provide valuable insights into the etiology and pathogenesis of PD.



Similarly with the mutations in the presenilin genes in patients with early onset Alzheimer's disease, the mutation identified in the alpha synuclein gene is unlikely to account for the majority of sporadic and familial cases of PD.

5 However, this mutation may account for a significant proportion of those families with a highly penetrant, early onset autosomal dominant PD phenotype.

All publications and patent applications herein are
10 incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

3. Summary of the Invention

15 As described herein, we have discovered that particular mutations in the alpha synuclein gene are associated with predisposition to Parkinson's disease. Accordingly, the present invention includes an isolated nucleic acid comprising a mutated synuclein gene. In particular, the
20 isolated nucleic acid of the present invention contains at least one mutation in the alpha synuclein gene at base pair position 209 of Genbank # L08850, which, in particular, is a change from guanine to adenine. However, since other mutations in the alpha synuclein gene may also lead to
25 Parkinson's Disease (PD), other mutations are also included. In addition, it is conceivable that mutations in the related beta (46) (SEQ ID NO 11) and gamma (SEQ ID NOs 12 and 13)



synuclein genes may also lead to PD. Thus, mutated
homologues of the alpha synuclein gene are also included in
the present invention. Vectors comprising the isolated
nucleic acid and host cells comprising such vectors are
5 included as well.

Knowledge of particular genes that are associated with
PD allows for the search for other specific PD mutations.
Accordingly, the present invention also includes a method of
using a synuclein gene sequence to identify specific PD
10 mutations. Such mutations may occur in an unrelated
population or in a family that demonstrates passage of PD
within the family tree.

Since knowledge of mutations associated with Parkinson's
disease allows the development of genetic screens that test
15 for an individual's chances of being predisposed to the
disease, and such tests may be performed by hybridization
analysis using oligonucleotides complementary to the sequence
of interest or by PCR amplification using oligonucleotides
that are complementary to sequences flanking the mutation,
20 the present invention also includes oligonucleotides
complementary to a portion of the synuclein gene, wherein
said portion comprises or flanks a mutation associated with
predisposition to Parkinson's Disease. In particular, the
oligonucleotides of the present invention will have a
25 sequence that is complementary to a sequence from the alpha
synuclein gene that includes or flanks base pair position
209. And in particular, this mutation is a change from
guanine to adenine at this position.



Vectors comprising an isolated nucleic acid encoding a mutated synuclein gene will allow the production and isolation of the mutant protein in an appropriate host cell using techniques well known in the art. Alternatively, peptides may be chemically synthesized using techniques also well known in the art. Isolation of such a protein or peptides thereof will allow the study of the molecular mechanisms which lead to development of Parkinson's disease. Accordingly, the present invention also includes an isolated synuclein protein or peptide containing at least one mutation. In particular, this mutation is at a position corresponding to the fifty-third amino acid in the native alpha synuclein protein, and in particular, this mutation is an alanine to threonine substitution.

Peptides corresponding to portions or the entirety of a synuclein gene may be useful as drugs for inhibiting the self-aggregation of mutant proteins that is thought to lead to Parkinson's disease. Accordingly, the present invention includes a method of testing peptides and other compounds for the ability to interfere with this self-aggregation. Self-aggregation can be tested using a number of established methods, including Congo red staining, electron microscopy pictures of amyloid fibrils, and circular dichroism (CD) spectrophotometry. Using a peptide derived from the alpha synuclein protein that includes the mutant THR amino acid at position 53 alone or in combination with a normal peptide may allow testing for drugs that can inhibit the aggregation or dissolve an aggregate. This procedure can be used to rapidly



identify agents that could be used in animal studies, clinical trials, or as diagnostic tools.

Possession of isolated synuclein proteins or peptides will also allow the isolation of specific antibodies using techniques well known in the art. Such antibodies may distinguish a mutant synuclein protein from its wildtype counterpart, and therefor could also be used in diagnostic screens. Alternatively, such antibodies may also be used to inhibit the self-aggregation of proteins during the progression of Parkinson's disease. Accordingly, the present invention also includes antibodies specific for a mutated synuclein protein or peptide. It should be understood that useful derivatives of such antibodies, such as Fv fragments and Fab fragments, are also included.

The above aspects of the present invention will allow methods of detecting subjects at increased risk for Parkinson's Disease. Such a method comprises obtaining a sample comprising nucleic acids from the subjects, and detecting in the nucleic acids the presence of a mutation which is associated with Parkinson's disease. In particular, the mutation detected by the method of the present invention is located on human chromosome four, preferably in the alpha synuclein gene. In particular, the mutation causes an amino acid substitution at position 53 of the alpha synuclein gene, which is, in particular, an alanine to threonine substitution.

The detecting step of the method of the present invention may be accomplished several different ways as will



be described in further depth below. All such methods are well known to those of ordinary skill in the art.

For instance, said detecting step may comprise combining a nucleotide probe which selectively hybridizes to a nucleic acid containing a mutation associated with a predisposition to Parkinson's disease, and detecting the presence of hybridization. Such a probe may be an oligonucleotide that is complementary to a portion of the synuclein gene, wherein said portion comprises the mutation. In particular, such an oligonucleotide is complementary to a mutated alpha synuclein gene having at least one mutation at base pair position 209. In particular, this mutation is a change from guanine to adenine.

The detecting step of the method of the present invention may also comprise amplifying a nucleic acid product comprising said mutation, and detecting the presence of said mutation in the amplified product using any nucleic acid sequencing procedure known in the art. Alternatively, the detecting step may comprise selectively amplifying a nucleic acid product comprising said mutation, and detecting the presence of amplification using any appropriate method known in the art. Such methods include gel electrophoresis of amplified nucleic acids, and detection of radiolabeled amplified nucleic acids using autoradiographic film or any other detection method known in the art.

The amplifying step of the present invention may be performed using the polymerase chain reaction (PCR), reverse transcriptase PCR (RTPCR), or any other type of PCR reaction



known in the art. Accordingly, such a step will comprise at least one annealing step whereby at least one oligonucleotide is annealed to said sample of nucleic acids. In particular, said amplifying step uses two oligonucleotides. And in particular, the two oligonucleotides have the sequences given in SEQ ID NOs 2 and 3.

Alternatively, the detecting step of the method of the present invention comprises detecting the presence or absence of a restriction endonuclease site as detected by enzymatic digest of a nucleic acid sample. Such a detecting means will be possible when a mutation associated with a predisposition to Parkinson's disease results in a sequence having a new restriction endonuclease cleavage site, or loss of a native restriction endonuclease site. In particular, the mutation associated with Parkinson's disease results in the formation of a non-native *Tsp45I* restriction endonuclease site.

Alternatively, the detecting step of the present invention may be performed using a gene-specific primer and subsequent chain termination at the position of the mutation using DNA polymerase and labeled nucleotides or dideoxynucleotides. The presence of nucleic acids in which a dideoxynucleotide corresponding to the mutation of interest is incorporated at the appropriate position may be detected by any means known in the art, including detection of radiolabeled dideoxynucleotides using, for example, autoradiographic film, or detection of fluorescently-labeled dideoxynucleotides.

Since the methods and compounds of the present invention



will be useful in diagnostic screening procedures aimed at identifying individuals having a predisposition for Parkinson's disease, the present invention also includes diagnostic kits which include the compounds of the present invention in a form that allows such compounds to be used quickly and easily for the designated purpose.

Finally, the inventors also contemplate that the isolated nucleic acid, oligonucleotides and antibodies of the present invention may eventually be used in methods directed at the correction or suppression of Parkinson's disease. For example, oligonucleotides or expression vectors designed from the synuclein nucleic acid sequences of the present invention may one day be used in antisense therapy directed at inhibiting expression of the mutated synuclein protein in patients with Parkinson's disease, or in individuals having a predisposition for Parkinson's disease. Similarly, antibodies specific for the mutated synuclein protein may be useful in therapies directed at inhibiting the self-aggregation of mutated proteins or peptides in patients having Parkinson's disease. Knowledge of gene(s) associated with the development of Parkinson's disease may also allow the design of transgenic animals which express the mutant gene(s). Such animals may serve as a useful disease model, allowing one to test the effects of candidate therapies and therapeutic compositions in the treatment or inhibition of Parkinson's disease.



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A detailed description of the present invention is now provided, and should not be considered as limiting on the present invention as described above.

4. Brief Description of the Drawings

Figure 1.

DNA sequence of the PCR product used for mutation detection (SEQ ID NO 1). Oligonucleotide primers are shown by arrows and the numerals 3 and 13 (SEQ ID NO 2 and 3).

Intron sequence is shown in lower case and exon sequence in upper case. Amino acid translation of the exon is shown below the DNA sequence. The circled base represents the G209A change in the mutant allele. The resulting amino acid Ala53Thr change is represented by the circled amino acid. The newly created *Tsp45* I site is indicated above the DNA sequence.

Figure 2.

Mutation analysis of the G209A change is shown in a subpedigree of the Italian kindred. Filled symbols represent affected individuals. Numerical identifiers, denote the individuals immediately above. *Tsp45* I digestion of PCR products is shown at the bottom of the figure, and fragment sizes are indicated on the right in base pairs.

Figure 3.



Mutation analysis of the G209A change in RT PCR products (7). Lane 1: 100 bp ladder, lanes 2 and 3 normal control, lanes 4 and 5 PD patient, lane 6 negative control without RT enzyme. Sizes are indicated on the right in base pairs. Lanes 2 and 4 show uncut DNA and lanes 3 and 5 show DNA cut with *Tsp45* I.

Figure 4.

Sequence alignments of alpha synuclein homologues in different species. Accession numbers for the sequences used were as follows: Homo sapiens Swiss-Prot P37840 (SEQ ID NO 4), Rattus norvegicus Swiss-Prot P37377 (SEQ ID NO 5), Bos taurus Swiss-Prot P33567 (SEQ ID NO 6), Serinus canaria genbank L33860 (SEQ ID NO 7), Torpedo californica Swiss-Prot P37379 (SEQ ID NO 8). Numbering on top of the alignments is according to the human sequence. Amino acid 53, which is the site of the Ala53Thr change, is circled.

Figure 5.

The pedigree of a large family with PD (3). The clinical and pathological features of some members of this kindred were previously reported.

Figure 6.

Multipoint LOD score analysis between chromosome 4q markers and the PD locus.



Figure 7.

A table of human synuclein clones identified from various databases. Columns labeled 5' and 3' show the sequence acquisition numbers. Clones were identified by
5 homology to protein or nucleic acid sequence. Human gamma clones were identified by homology to known mouse and rat gamma synuclein sequences.

Figure 8.

10 Sequence of BAC clone 139A20 for human beta synuclein. BAC clone was isolated using primers to known database sequences described in Figure 7. The sequence shown includes all coding exon sequences and some non-coding intronic sequences. (SEQ ID NO:11)

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Figure 9.

Sequence from the 5' end of BAC clone 174P13 for human gamma synuclein. The BAC clone was isolated with primers from the database sequences described in Figure 7. (SEQ ID
20 NO:12)

Figure 10.

Sequence from the 3' end of BAC clone 174P13 for human gamma synuclein. BAC clone was isolated as described in
25 Figure 9. The entire human gamma synuclein gene has now been sequenced and has been deposited in GenBank: accession number



AF044311.(SEQ ID NO: 13)

Figure 11.

Sequence of exons 1-7 of the human alpha synuclein gene,
plus some flanking intronic sequence for each exon.(SEQ ID
5 NOS 14-19)

5. Detailed Description of the Invention

Definitions

Unless defined otherwise, all technical and scientific
10 terms used herein have the same meaning as commonly
understood by one of ordinary skill in the art to which this
invention belongs. Although any methods and materials similar
or equivalent to those described herein can be used in the
practice or testing of the present invention, the preferred
15 methods and materials are described. For purposes of the
present invention, the following terms are defined below.

This invention provides a method of diagnosing or
predicting a predisposition to Parkinson's disease. The
method comprises detecting in a sample from a subject the
20 presence of a mutation, for example, in nucleotide position
209 of the human alpha synuclein gene. The presence of the
mutation indicates the presence of or a predisposition to
Parkinson's disease.

As used herein, the term "gene" primarily relates to a
25 coding sequence, but can also include some or all of the
surrounding or flanking regulatory regions or introns. The
term "gene" specifically includes artificial or recombinant



genes created from cDNA or genomic DNA, including recombinant genes based upon splice variants.

As used herein, the term "synuclein" gene or protein may refer to the alpha synuclein gene or any homologue thereof.

5 A "homologue" is understood to mean any related gene or protein that is at least 25% homologous to the alpha synuclein gene or protein or performs a related function. Preferably, a synuclein gene or protein refers to alpha, beta or gamma synuclein, but most preferably refers to alpha
10 synuclein.

As used herein, an "isolated nucleic acid" is a ribonucleic acid, deoxyribonucleic acid, or nucleic acid analog comprising a polynucleotide sequence that has been isolated or separated from sequences that are immediately
15 contiguous, i.e. on the 5' and 3' ends, in the naturally occurring genome of the organism from which it is derived. The term therefor includes, for example, a recombinant nucleic acid which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the
20 genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule independent from any other sequences.

An isolated nucleic acid of the present invention may be "operatively linked" to an expression control sequence or regulatory region. As used herein, "operatively linked"
25 means that the components are joined in such a way that the expression, transcription or translation of the sequence is under the influence or control of the regulatory region.

As used herein, a "predisposition" to Parkinson's



disease means an increased probability of developing Parkinson's disease during the subject's lifetime as compared to the average individual.

Pertaining to this probability, a LOD score is a measure of genetic linkage used herein, defined as the \log_{10} ratio of the probability that the data would have arisen if the loci are linked to the probability that the data could have arisen from unlinked loci. The conventional threshold for declaring linkage is a LOD score of 3.0, that is, a 1000:1 ratio (which must be compared with the 50:1 probability that any random pair of loci will be unlinked).

As used herein, reference to "base pair position" or "amino acid position" when referring to an isolated nucleic acid, probe, protein or peptide always indicates the relative position in the native gene or protein.

A "probe" refers to a nucleic acid which has sufficient nucleotides surrounding the codons at the mutation positions to distinguish the nucleic acid from nucleic acids encoding non-related genes. The specific length of the nucleic acid is a matter of routine choice based on the desired function of the sequence. For example, if one is making probes to detect the mutation in base pair position 209, the length of the nucleic acid is preferably small, but must be long enough to prevent hybridization to undesired background sequences. However, if the desired hybridization is to a nucleic acid which has been amplified, background hybridization is less of a concern and a smaller probe can be used. In general, such a probe will be between 10 and 100 nucleotides, especially



between 10 and 40 and preferably between 15 and 25 nucleotides in length. It is apparent to one of skill in the art that nucleotide substitutions, deletions, and additions may be incorporated into the polynucleotides of the invention. However, such nucleotide substitutions, deletions, and additions should not substantially disrupt the ability of the polynucleotide to hybridize under conditions that are sufficiently stringent to result in specific hybridization.

As used herein with respect to genes, "the term "normal" refers to a gene which encodes a normal protein. As used herein with respect to proteins, the term "normal" means a protein which performs its usual or normal physiological role and which is not associated with, or causative of, a pathogenic condition or state. Therefor, the term "normal" is generally synonymous with the phrase "wild type".

For any given gene or corresponding protein, a multiplicity of normal allelic variants may exist, none of which is associated with the development of a pathogenic condition or disease state. Such normal allelic variants include, but are not limited to, variants in which one or more nucleotide substitutions do not result in a change in the encoded amino acid sequence.

As used herein, the term "mutation" generally refers to a mutation in a gene that is associated with a predisposition to Parkinson's disease. "Mutant" can specifically refer to a mutation at nucleotide position 209 of the synuclein gene, and is in particular a G to A transition. However, other mutations in the synuclein gene or other genes which are



associated with a predisposition to Parkinson's disease are also encompassed. Furthermore, the term "mutation" is not limited to transition mutations, but can also mean a deletion, insertion or transversion as well.

5 The term "mutant", as it applies to synuclein genes, is not intended to embrace sequence variants which, due to the degeneracy of the genetic code, encode proteins identical to the normal sequences disclosed or otherwise enabled herein; nor is it intended to embrace sequence variants which,
10 although they encode different proteins, encode proteins which are functionally equivalent to normal synuclein proteins. The term "mutant" means a protein which does not perform its usual or normal physiological role and which is associated with, or causative of, a pathogenic condition or
15 state.

 Since a mutation can be a substitution, deletion or insertion, a mutated synuclein "protein" is understood to refer to the amino acid sequence resulting from any such mutation whether the resulting protein is shorter, longer or
20 modified, i.e. due to an alteration in reading frame or generation of stop codon. In addition, "peptide" is understood to refer to a portion of the mutated protein that is preferably at least five base pairs long, and more preferably at least 10 base pairs long. This portion may be
25 derived from the amino or carboxyl terminus, or it may be an internal portion of the full length protein. As such, a peptide may be chemically synthesized using any method known in the art, or may be made using a recombinant DNA technology



and an appropriate purification scheme or isolated from the native protein using enzymatic digestion.

As used herein, the term "substantially pure" means a preparation which is at least 60% by weight the compound of interest. Preferably the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate method, i.e. column chromatography, gel electrophoresis or HPLC analysis.

"Specific or selective hybridization" as used herein means the formation of hybrids between a probe nucleic acid (e.g., a nucleic acid which may include substitutions, deletions, and/or additions) and a specific target nucleic acid (e.g., a nucleic acid having the mutated sequence), wherein the probe preferentially hybridizes to the specific target such that, for example, a band corresponding to the mutated DNA or restriction fragment thereof can be identified on a Southern blot, whereas a corresponding normal or wild-type DNA is not identified or can be discriminated from a variant DNA on the basis of signal intensity. Hybridization probes capable of specific hybridization to detect a single-base mismatch may be designed according to methods known in the art (13-17).

"Stringent" as it refers to hybridization conditions is a term of art understood by those of ordinary skill to refer to those conditions of temperature, chaotropic acids, buffer and ionic strength which permit hybridization of a particular nucleic acid sequence to its complementary sequence and not



to substantially different sequences. The exact conditions which constitute "stringent" conditions depend on the nature of the nucleic acid sequence, the length of the sequence, and the frequency of occurrence of subsets of that sequence

5 within other non-identical sequences. By varying hybridization conditions from a level of stringency at which non-specific hybridization occurs to a level at which only specific hybridization occurs, one of ordinary skill in the art can, without undue experimentation, determine conditions
10 which will allow a given sequence to hybridize only with complementary sequences.

Suitable ranges of stringency conditions are described in Sambrook et al. (13). Hybridization conditions, depending on the length and commonality of a sequence, may include
15 temperatures of 20°C-65°C and ionic strengths from 5X to 0.1X SSC. Highly stringent hybridization conditions may include temperatures as low as 40°C-42°C (when denaturants such as formamide are included) or up to 60°C-65°C in ionic strengths as low as 0.1X SSC. These ranges are, however, only
20 illustrative and, depending on the nature of the target sequence, and possible future technological developments, may be more stringent than necessary. Appropriate conditions may be determined for each specific nucleic acid sequence or oligonucleotide probe using standard control and a level of
25 experimentation that is not considered to be undue by those of skill in the art.



As discussed below in greater detail, the mutation can be detected by many methods. For example, the detecting step can comprise combining a nucleotide probe capable of selectively hybridizing to a nucleic acid containing the mutation with a nucleic acid in the sample and detecting the presence of hybridization. Additionally, the detecting step can comprise amplifying the nucleotides surrounding and including the mutation and detecting the presence of the mutation in the amplified product, or selectively amplifying the nucleotides of the mutation and detecting the presence of amplification. Finally, the detecting step can comprise detecting the presence or absence of a restriction fragment created by an enzyme digest of the sample nucleic acid, or any other detection means known in the art.

Detection Techniques

Once the location of a PD-relevant mutation is known, the methods to detect such a mutation are standard in the art. The sequence of various nucleotide probes can be determined from the known sequence of the relevant gene, especially the sequences surrounding the mutation.

Detection of point mutations using direct probing involves the use of oligonucleotide probes which may be prepared, for example, synthetically or by nick translation. The probes may be suitably labeled using, for example, a radio label, enzyme label, fluorescent label, biotin-avidin label and the like for subsequent visualization by any appropriate assay, i.e. Southern blot hybridization. In this procedure, the labeled



probe is reacted with sample DNA that is bound, for example, to a nylon filter under conditions such that only fully complementary sequences hybridize. The areas that carry DNA sequences complementary to the labeled DNA probe become
5 labeled themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling may then be visualized, for example, by autoradiography.

Methods of manipulating hybridization conditions to achieve varying degrees of specificity are well known in the
10 art. For example, tetra-alkyl ammonium salts may be used to bind selectively to A-T base pairs, thus displacing the dissociation equilibrium and raising the melting temperature. At 3M Me 4NCl, this is sufficient to shift the melting
15 temperature to that of G-C pairs. This results in a marked sharpening of the melting profile. The stringency of hybridization in such an experiment is usually 5°C below the T_i (the irreversible melting temperature of the hybrid formed between the probe and its target sequence) for the given
20 chain length. For a 20mer oligonucleotide probe, the recommended hybridization temperature is about 58°C. The washing temperatures are unique to the sequence under investigation and need to be optimized for each variant.

There are certainly other ways known in the art for adjusting hybridization conditions in view of desired
25 specificity. For instance, although hybridization may be carried out in accordance with conventional hybridization methods under suitable conditions with respect to e.g. stringency, incubation time, temperature, etc, the choice of



conditions will depend on the desired degree of complementarity between the fragments to be hybridized. A high degree of complementarity requires more stringent conditions such as low salt concentrations, low ionic strength of the buffer and higher temperatures, whereas a low degree of complementarity requires less stringent conditions, e.g. higher salt concentration, higher ionic strength of the buffer or lower temperatures, for the hybridization to take place.

The support to which DNA or RNA fragments of the sample to be analyzed are bound in denatured form is preferably a solid support and may have any convenient shape. Thus, it may, for instance, be in the form of a plate, e.g. a thin layer or a microtiter plate, a strip, a solid particle e.g. in the form of a bead such as a latex bead, a filter, a film or paper. The solid support may be composed of a polymer, preferably nylon or nitrocellulose.

Alternative probing techniques, such as ligase chain reaction (LCR), may involve the use of mismatch probes, i.e., probes which are fully complementary with the target except at the point of the mutation. The target sequence is then allowed to hybridize both with oligonucleotides which are fully complementary and have oligonucleotides containing a mismatch, under conditions which will distinguish between the two. By manipulating the reaction conditions according to the above considerations, it is possible to obtain hybridization only where there is full complementarity. If a mismatch is present there is significantly reduced hybridization.



The polymerase chain reaction (PCR) is a technique that amplifies specific DNA sequences with remarkable efficiency. Repeated cycles of denaturation, primer annealing and extension carried out with Taq polymerase, e.g., a heat

5 stable DNA polymerase, leads to exponential increases in the concentration of desired DNA sequences. Given a knowledge of the nucleotide sequence of the mutations, synthetic oligonucleotides can be prepared which are complementary to sequences which flank the DNA of interest. Each

10 oligonucleotide is complementary to one of the two strands. The DNA is denatured at high temperatures (e.g., 95°C) and then reannealed in the presence of a large molar excess of oligonucleotides. The oligonucleotides, oriented with their 3' ends pointing towards each other, hybridize to opposite

15 strands of the target sequence and prime enzymatic extension along the nucleic acid template in the presence of the four deoxyribonucleotide triphosphates. The end product is then denatured again for another cycle. After this three-step cycle has been repeated several times, amplification of a DNA

20 segment by more than one million-fold can be achieved. The resulting DNA may then be directly sequenced in order to locate any genetic alteration. Alternatively, it may be possible to prepare oligonucleotides that will only bind to altered DNA, so that PCR will only result in multiplication

25 of the DNA if the mutation is present. Following PCR, direct visualization or allele-specific oligonucleotide hybridization (18) may be used to detect the Parkinson's disease point mutation. Alternatively, PCR may be followed



by restriction endonuclease digestion with subsequent analysis of the resultant products.

As shown in the examples, the substitution of G for A at base pair 209 of the synuclein, results in the gain of a
5 Tsp45I site. The gain of this restriction endonuclease recognition site facilitates the detection of the Parkinson's disease mutation using restriction fragment length polymorphism (RFLP) analysis or by detection of the presence or absence of the restriction site in a PCR product that
10 spans base pair position 209.

For RFLP analysis, DNA is obtained, for example from the blood cells of the subject suspected of having Parkinson's disease and from a normal subject, is digested with a restriction endonuclease, and subsequently separated on the
15 basis of size by agarose gel electrophoresis. The Southern technique can then be used to detect, by hybridization with labeled probes, the products of endonuclease digestion. The patterns obtained from the Southern blot can then be compared. Using such an approach, an additional restriction
20 endonuclease site, such as a Tsp45I site, is detected by determining the number of bands detected and comparing this number to the normal subject.

The creation of a new restriction site as a result of a nucleotide substitution at a disclosed mutation site can be
25 readily determined by reference to the genetic code and a list of nucleotide sequences recognized by restriction endonucleases (19).

In general, primers for PCR are usually about 20 bp in



length, and are most preferably 15-25 bp. Denaturation of strands usually takes place at 94°C. and extension from the primers is usually at 72°C. The annealing temperature varies according to the sequence under investigation. Examples of reaction times are: 20 mins denaturing; 35 cycles of 2 min, 1 min, 1 min for annealing, extension and denaturation; and finally a 5 min extension step.

PCR "amplification of specific alleles" (PASA) may also be used to detect the presence of the PD mutation. PASA is a rapid method of detecting single-base mutations or polymorphisms (22-28). PASA (also known as allele specific amplification) involves amplification with two oligonucleotide primers such that one is allele-specific. The desired allele is efficiently amplified, while the other allele(s) is poorly amplified because it mismatches with a base at or near the 3' end of the allele-specific primer. Thus, PASA or the related method of PAMSA may be used to specifically amplify the mutation sequences of the invention. Where such amplification is done on genetic material (or RNA) obtained from an individual, it can serve as a method of detecting the presence of the mutations.

As mentioned above, a method known as ligase chain reaction (LCR) can be used to successfully detect a single-base substitution (29, 30). LCR probes may be combined or multiplexed for simultaneously screening for multiple different mutations. Thus, LCR can be particularly useful where multiple mutations are predictive of the same disease.



Finally, the Parkinson's disease mutation of the present invention may also be detected using chain termination with labeled dideoxynucleotides. For instance, U.S. Patent No. 5,047,519 to Hobbs et al. discloses fluorescently-labeled nucleotides as chain-terminating substrates for a fluorescence-based DNA sequencing method. With such substrates and knowledge of the gene sequence of interest, it is possible to design an assay using a gene-specific primer to initiate a polymerase reaction immediately flanking the position of the mutation, employing color-coded dideoxynucleotide terminators such that the specific nucleotide at the position of the mutation may be easily determined via a colorimetric assay.

15 Transgenic Animals and Cell Lines

Having identified subjects having a predisposition to Parkinson's disease associated with a specific mutation, the subjects can participate in the screening of putative agents capable of treating Parkinson's disease. This method comprises administering the test agent to the subject, which may be a human, which has a mutation in a gene associated with Parkinson's disease and monitoring the effect of the agent on the subject's condition. If the symptoms of Parkinson's disease improve, the agent can be used as a treatment for the disease.

In addition, it is possible to develop transgenic model systems and/or cell lines containing the mutated nucleic acid(s) for use, for example, as model systems for screening



for drugs and evaluating drug efficiency. Additionally, such model systems provide a tool for defining the underlying biochemistry of, for instance, the mutated synuclein gene, thereby providing a rationale for drug design.

5 One approach to creating transgenic animals is to mutate the animal gene of interest by in vivo mutagenesis, transfer the mutant gene into embryonic stem cells by DNA transfection and inject the embryonic stem cells into blastocysts in order to retrieve offspring which carry the disease-causing
10 mutation (31). Alternatively, the technique of microinjection of the mutated gene, into a one-cell embryo followed by incubation in a foster mother can be used. Alternatively, viral vectors, e.g., Adeno-associated virus, can be used to deliver the mutated gene to a stem cell, or may be used to
15 target specific cells of a fully developed animal (32,33).

Antibodies and Recombinant Expression of Polypeptides

When the mutated gene product is a polypeptide, e.g. the 209 mutation, it can be used to prepare antisera and
20 monoclonal antibodies using, for example, the method of Kohler and Milstein (34). Such monoclonal antibodies could then form the basis of a diagnostic test, or may even be useful in therapies directed toward inhibiting the action of the mutant protein in a patient with Parkinson's disease.

25 Mutant polypeptides can also be used to immunize an animal for the production of polyclonal antiserum (35). For example, a recombinantly produced fragment of a variant polypeptide can be injected into a mouse along with an



adjuvant so as to generate an immune response. Murine immunoglobulins which specifically bind the recombinant fragment can be harvested from the immunized mouse as an antiserum, and may be further purified by affinity chromatography or other means. Additionally, spleen cells are harvested from the mouse and fused to myeloma cells to produce a bank of antibody-secreting hybridoma cells, which can then be screened for clones that secrete immunoglobulins which bind the recombinantly produced fragment with an increased affinity. More specifically, immunoglobulins that selectively bind to the variant polypeptides but poorly or not at all to wild-type polypeptides are selected, either by pre-absorption with wild-type proteins or by screening of hybridoma cell lines for specific idiotypes that bind the variant but not wild-type.

These antibodies can be used to screen protein and tissue samples for the presence of mutated proteins. A colored enzymatic reaction occurs when the specific antibody remains bound to its target protein, in situ, after thorough washing, as directed by established protocols.

Gene expression

The nucleic acid sequences of the present invention will be capable of expressing the desired mutant or normal polypeptides in an appropriate host cell. For expression in host cells, the DNA sequences of the present invention will be operably linked to, i.e., positioned to ensure the functioning of, an expression control sequence. For example,



such polynucleotides can include a promoter, a transcription termination site (polyadenylation site in eukaryotic expression hosts), a ribosome binding site, and, optionally, an enhancer for use in eukaryotic expression hosts. In addition, the DNA sequence of the present invention may also be fused such that the reading frame is conserved to an appropriate signal sequence to facilitate export of the encoded protein across the cell membrane.

Expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. A variety of suitable expression vectors are disclosed in Sambrook et al. (13). Commonly, expression vectors will contain selection markers, e.g., tetracycline resistance or hygromycin resistance, to permit detection and/or selection of those cells transformed with the desired DNA sequences.

E. coli is one prokaryotic host that is particularly useful for cloning and expression of the DNA sequences of the present invention because of the wide variety of available expression systems. Vectors suitable for use in *E. coli* are known and are commercially available, i.e. pBR322 (13), pBLUESCRIPT (Stratagene), etc. Also, a variety of different types of expression systems may be used, including plasmids, cosmids, bacteriophage lambda, etc. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. Expression vectors for use in prokaryotic host cells will typically contain expression



control sequences compatible with the host cell (e.g., an origin of replication). In addition, any of a variety of well-known promoters may be used, such as the lactose promoter system, a tryptophan (Trp) promoter system, a
5 beta-lactamase promoter system, or a promoter system from phage lambda. A promoter may optionally contain an operator sequence for regulatable gene expression, and will have a ribosome binding site sequence for the initiation of translation.

10 In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention (36). Vectors for use in eukaryotic cells are known and commercially available, i.e. pcDNA3 (Invitrogen). Eukaryotic cells are actually
15 preferred, and a number of suitable host cell lines capable of secreting intact human proteins have been developed in the art, including CHO cells, COS cells, HeLa cells, myeloma cell lines, Jurkat cells, etc. Promoters for use in eukaryotic vectors may be cell-specific, or capable of being expressed
20 in a wide variety of cells, i.e. viral promoters.

Expression vectors of the present invention (e.g., comprising nucleic acid sequences encoding a mutant or normal polypeptide) can be transferred into the host cell by well-known methods, which vary depending on the type of
25 cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts.



Kits

The method lends itself readily to the formulation of test kits which can be utilized in diagnosis. Such a kit would comprise a carrier compartmentalized to receive in
5 close confinement one or more containers wherein a first container may contain suitably labeled DNA probes. Other containers may contain reagents useful in the localization of the labeled probes, such as enzyme substrates. Still other containers may contain restriction enzymes (such as *Tsp45I*),
10 buffers, etc., together with instructions for use.

DESCRIPTION OF THE INVENTIONDetailed Description of the Preferred Embodiments

The following laboratory procedures were used:

15 DNA samples were collected upon informed consent. High molecular weight genomic DNA was isolated from whole-blood lysate by methods previously described (38). Genotyping was performed as previously described (39). Pairwise linkage analysis was performed using the MLINK program of the
20 FASTLINK package (40-42). Allele frequencies were used as reported in the Genomic Data Base (<http://gdbwww.gdb.org>) and the Cooperative Human Linkage Consortium (CHLC) database (<http://www.chlc.org>). Multipoint analysis was performed using the LINKMAP program of the FASTLINK package. For the
25 multipoint analysis allele frequencies were set to $1/n$ where n equals the number of alleles observed. In the two point analysis LOD scores were calculated for both the reported and the $1/n$ allele frequencies with minimal effect on the



maximum LOD score observed. Simulations of multipoint analysis in a subset of the pedigree with different allele frequencies similarly indicated no significant effect on the scores calculated. Maximum LOD scores as shown were observed for the heterozygote and homozygote disease allele penetrance set to 0.99, which is similar to the PD allele penetrance previously reported ranging from 0.88 to 0.94 (3). All unaffected individuals used in the study were of age above the mean for onset of illness. Disease allele frequency was set to 0.0001. Distances and order of genetic markers were set as reported in the CHLC database. Overlapping three point analysis was performed for markers *D4S2361*, *D4S1647*, *D4S421* and the PD locus. The 12 allele *D4S2380* locus was not included because of prohibitive time run. Multipoint analysis was performed on an IBM SP2 parallel computer and the SGI Challenge machine.

For mutation analysis genomic DNA was amplified with oligonucleotides (3): 5' GCTAATCAGCAATTTAAGGCTAG 3' (SEQ ID NO 2) and (13): 5' GATATGTTCTTAGATGCTCAG 3' (SEQ ID NO 3) of genbank ID: U46898, under standard PCR conditions. Sequence analysis was performed using the Perkin Elmer dye terminator cycle sequencing kit on an ABI 373 fluorescent sequencer (ABI, Foster City, CA). Restriction digestion was performed following the PCR with *Tsp45 I* according to manufacturer's protocol (New England Biolabs, Beverly, MA). The digested PCR products were electrophoresed on a 6% Visigel (Stratagene, La Jolla, CA), and visualized by ethidium



bromide staining. Pedigree structure in Figure 2 has been slightly modified in order to protect patient confidentiality.

Total RNA was extracted from the lymphoblastoid cell line of an affected individual and first strand synthesis was performed by oligo dT priming (Gibco BRL, Gaithersburg, MD). Primers (1F) 5' ACGACAGTGTGGTGTAAGG 3' (SEQ ID NO 9) and (13R) 5' AACATCTGTCAGCAGATCTC 3' (SEQ ID NO 10) corresponding to nucleotides 21-40 and 520-501 of genbank L08850 were used to amplify a product of 500 bp containing the mutation at nucleotide 209. PCR products were subjected to restriction digestion by *Tsp45 I*. The mutation at nt 209 creates a novel *Tsp45 I* site (Figure 1), so that the normal allele will be restricted in 4 fragments of 249, 218, 24 and 9 bp, where the mutant allele will have 5 fragments of 249, 185, 33, 24 and 9 bp of size, as shown in Figure 3. Size standards used, were the 100 bp ladder (Gibco BRL, Gaithersburg, MD).

Example 1

In an effort to identify a genetic locus responsible for Parkinson's disease, we performed a genome scan in a large kindred of Italian descent with pathologically confirmed PD (Figure 5). The kindred originated in the town of Contursi in the Salerno province of Southern Italy (3). Some members emigrated to the United States, Germany and other countries. The extended family pedigree consists of 592 members with 60 individuals affected by PD. The average age of onset for the



illness in this pedigree (Figure 5) has been shown to be 46 ± 13 years. One hundred and forty genetic markers were typed in this pedigree at an average spacing of about 20 cm. Genetic markers at the cytogenetic location 4q21-q23 were the only ones to show linkage to the disease phenotype with a $Z_{\max}=6.00$ at $\theta=0.00$ for marker *D4S2380I* (see Table 1).

Table 1. Two point LOD scores between chromosome 4q markers and the PD locus

Locus	Two-point LOD scores at recombination fractions of:								Z_{\max}	θ_{\max}
	0.00	0.01	0.05	0.10	0.20	0.30	0.40			
<i>D4S2361</i>	-5.60	-0.83	0.30	0.54	0.43	0.21	0.06		0.55	0.12
<i>D4S2380</i>	6.00	5.90	5.30	4.60	3.00	1.50	0.50		6.00	0.00
<i>D4S1647</i>	5.22	5.07	4.47	3.71	2.26	1.05	0.30		5.22	0.00
<i>D4S421</i>	-2.42	0.45	0.77	0.65	0.38	0.22	0.09		0.77	0.05

Recombinations between the disease phenotype and genetic markers were observed in the proximal region for marker *D4S2361* and in the distal region for marker *D4S421*. Genetic markers *D4S2380* and *D4S1647* showed no obligate recombination events in the affected individuals.

Multipoint LOD score analysis between markers *D4S2361*-13cM-*D4S1647*-3cM-*D4S421* and the disease locus places the PD gene between markers *D4S2361* and *D4S421* at a recombination distance of 0.00 cM from marker *D4S1647* with a $Z_{\max}=6.04$ (Figure 6). This location is favored from the alternative genetic intervals by a difference in the LOD score of greater



than three LOD units.

Although expansions of unstable trinucleotide repeats are found in a number of human neurodegenerative conditions, there is no evidence for an association of a CAG

5 trinucleotide repeat expansion in families with PD (43). In addition, genetic linkage studies in other families with PD-like illnesses do not support the involvement of several candidate genes (glutathione peroxidase, tyrosine hydroxylase, brain-derived neurotrophic factor, catalase,

10 amyloid precursor protein, CuZn superoxide dismutase and debrisoquinone 4-hydroxylase) in the etiology of the disorder (44). Genes previously mapped in the general region of linkage include the loci for alcohol dehydrogenase, formaldehyde dehydrogenase, synuclein, UDP-N-

15 acetylglycosamine phosphotransferase and others.

Our localization of a PD susceptibility gene represents the first genetic locus linked to PD. Other distinct clinicopathological entities associated with parksonian features are probably linked to other genetic loci. For

20 example, Wilhelmsen-Lynch disease (disinhibition-dementia-parkinsonian-amyotrophy complex) is linked to the 17q21-q22 chromosomal region (45). If the pathogenesis of diseases affecting the nigrostriatal pathway includes environmental influences, then a range of mutations affecting vulnerable

25 sites in the electron transport chain or enzyme polymorphisms influencing neurotoxin metabolism may vary the penetrance of PD by altering an individual's resistance to exogenous or endogenous agents. However, our finding of a highly



penetrant genetic locus linked to PD suggested that abnormalities of a single gene may be sufficient to cause Parkinson's disease.

5 **Example 2**

In an effort to identify a specific gene between markers *D4S2361* and *D4S421* that is associated with predisposition to Parkinson's disease, we conducted sequence analysis of candidate genes in this region.

10 Alpha synuclein, a presynaptic nerve terminal protein, was originally identified as the precursor protein for the NAC peptide, a non beta amyloid component of Alzheimer's disease (AD) amyloid plaques (4). The human alpha synuclein gene was previously mapped in the 4q21-q22 region (5). We
15 refined the mapping, and determined that the alpha synuclein gene is located within the non-excluded region harboring the PD gene in the Italian kindred. Thus alpha synuclein represented an excellent candidate locus for PD.

20 Sequence analysis of the fourth exon of the alpha synuclein gene revealed a single base pair G209A change from the published sequence of the gene (GenBank ID L08850), which results in an Ala53Thr substitution and the creation of a novel *Tsp45* I restriction site (Figure 1). Mutation analysis
25 for the G209A change in the Italian kindred shows complete segregation with the PD phenotype with exception of



individual 30 (Figure 2), who is affected but not carrying this mutation. This individual apparently inherited a different PD mutation from his father, as we have shown that he shares a genetic haplotype with his unaffected maternal
5 uncle, individual 3, for genetic markers in the PD linkage region.

The frequency of this variation was studied in two general population samples, one consisting of 120 chromosomes of the parents of the CEPH reference families, and the other
10 consisting of 194 chromosomes of unrelated individuals from the blood bank in Salerno, Italy, a city near the town from which the family originated. Of these 314 general population chromosomes none was found to carry the G209A mutation. Fifty two patients of Italian descent with sporadic PD were
15 also screened for the mutation (Figure 2), along with 5 probands from previously unpublished Greek families with PD. The Ala53Thr change was found to be present in three of the Greek kindreds and it segregated with the PD phenotype. In those three Greek kindreds it is worth noting that the age of
20 onset for the disease is relatively early, ranging from the mid 30's to the mid 50's. Extended haplotype analysis of the Greek kindreds and the Italian PD family suggests that the mutations arose independently on different ancestral chromosomes. The finding of the Ala53Thr substitution in
25 four independent PD families and its absence from 314 control chromosomes provides the strongest genetic evidence that this



mutation in the human alpha synuclein gene is causative for the PD phenotype in these families.

We have also demonstrated by RT PCR that the mutant allele is transcribed in the lymphoblast cell line of an affected individual from the Italian kindred (Figure 3) (7). Thus, it is reasonable to assume that the mutant protein is indeed expressed.

Example 3.

Since homologous genes that are related to the alpha synuclein protein have been identified in other species, it seemed reasonable to assume that homologues of alpha synuclein would exist in humans as well. In fact, human beta synuclein has previously been described (46), and is approximately 60% similar to alpha synuclein at the protein level.

We set out to identify other related homologues by searching various databases for homologous genes and proteins. Protein sequence databases searched included the NR (non-redundant) and "month" databases of Genbank and Swiss Prot. Nucleotide databases included NR, month, dbstf, GSS (Genome Sequence Service) and EPD (eukaryotic Promoter Database). Several human clones were identified and characterized as alpha, beta and gamma clones as shown in Figure 7. Potential gamma clones were identified on the basis of homology to known rat and mouse sequences. Although



gamma synuclein has been identified in species other than human, this is the first identification of the corresponding gamma synuclein from humans.

Using two primers sets designed from known database
5 sequences (5'ATGTCTTCAAGAAGGGCTTC3'; 5'CCTTGGTCTTCTCAGCTGCT3'
and 5'AGCGTGGATGACCTGAAGAG3'; 5'AGCACAGGTGGACAGGCCAAG3'), we
have isolated two BAC clones, 139A20 and 174P13, from a
Genome System commercial Bacterial Artificial Chromosome
library (St. Louis, MO) which contain the human beta and
10 gamma synuclein genes, respectively. The beta gene contained
one clone 139A20 has been sequenced as shown in Figure 8 (SEQ
ID NO 11), which contains all coding exon sequences and some
additional non-coding intronic sequence. The gamma clone
174P13 has been sequenced and is available in GenBank:
15 accession number AF044311. Sequence from the 5' end is given
in Figure 9 (SEQ ID NO 12), and sequence from the 3' end is
given in Figure 10 (SEQ ID NO 13). The human alpha
synuclein gene has also been sequenced as shown in Figure 11,
which provides the sequence of each separate exon region with
20 some additional flanking intronic sequence for each exon.
(SEQ ID NOs 14-19)

The three human homologues are highly conserved at the
protein level. The alpha and beta human homologues have
about 60.4% similarity. And the gamma homologue is about
25 38.3% and 32.8% similar to the alpha and beta homologues,
respectively, based on the portion of the coding sequence



that we have obtained thus far. Thus, it is reasonable to presume that mutations in either the beta or gamma synuclein gene may also result in Parkinson's disease.



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47. Polymeropoulos et al. (1997) Science 276:2045-2047, which
is relied upon and hereby expressly incorporated by reference
5 herein.

48. Lavedan et al. (1998) in press, which is relied upon and
hereby expressly incorporated by reference herein.

49. This application is based on provisional application
number 60/505,684 filed June 25, 1997 which is relied upon
10 and hereby expressly incorporated by reference herein.



SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT: Polymeropoulos, Mihael
Lavedan, Christian
Leroy, Elisabeth
Nussbaum, Robert
10 Johnson, William
Duvoisin, Roger

(ii) TITLE OF INVENTION: Cloning of a gene mutation for
Parkinson's disease

15

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:

20

(A) ADDRESSEE: SPENCER & FRANK
(B) STREET: 1100 New York Ave. Suite 300 East
(C) CITY: Washington
(D) STATE: D.C.
(E) COUNTRY: USA
(F) ZIP: 20005-3955

25

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
30 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

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(A) APPLICATION NUMBER:
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(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Schneller, John W.
(B) REGISTRATION NUMBER: 26,031
40 (C) REFERENCE/DOCKET NUMBER: NIH 0082A

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (202)414-4000
45 (B) TELEFAX: (202)414-4040

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS.

50

(A) LENGTH: 216 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear



(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

5 (iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(B) CLONE: alpha synuclein gene/ exon 4 region

10

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

20 GCTAATCAGC AATTAAAGGC TAGCTTGAGA CTTATGTCTT GAATTTGTTT TTGTAGGCTC 60
CAAAACCAAG GAGGGAGTGG TGCATGGTGT GACAACAGGT AAGCTCCATT GTGCTTATAT 120
CAAAGATGAT ATNTAAAGTAT CTAGTGATTA GTGTGGCCCA GTATCAAGAT TCCTATGAA 181
25 ATTGTAAAACA ATCACTGAGC ATCTAAGAAC ATATC 216

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

30

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "primer #3"

(iii) HYPOTHETICAL: NO

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

45 GCTAATCAGC AATTTAGGCT AG 22

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

50

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear



(ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "primer #13"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTATACAAGA ATCTACGAGT C

21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 140 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens
 (C) INDIVIDUAL ISOLATE: Swiss-Prot P37840

(vii) IMMEDIATE SOURCE:

(B) CLONE: alpha synuclein protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
 1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
 20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45

Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
 50 55 60

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
 65 70 75 80

Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys
 85 90 95



Lys Asp Gln Leu Gly Lys Asn Clu Glu Gly Ala Pro Gln Glu Gly Ile
 100 105 110

5 Leu Glu Asp Met Pro Val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro
 115 120 125

Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
 130 135 140

10 (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 140 amino acids
 (B) TYPE: amino acid
 15 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

20 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

25 (A) ORGANISM: Rattus norvegicus
 (C) INDIVIDUAL ISOLATE: Swiss-Prot P37377

(vii) IMMEDIATE SOURCE:

30 (B) CLONE: alpha synuclein protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

35 Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
 1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
 20 25 30

40 Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45

45 Val His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
 50 55 60

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
 65 70 75 80

50 Thr Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Phe Val Lys
 85 90 95

Lys Asp Gln Met Gly Lys Gly Glu Glu Gly Tyr Pro Gln Glu Gly Ile
 100 105 110



Leu Glu Asp Met Pro Val Asp Pro Ser Ser Glu Ala Tyr Glu Met Pro
 115 120 125

5 Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
 130 135 140

(2) INFORMATION FOR SEQ ID NO:6:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 134 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: not relevant

15 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

20 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Bos taurus
 (C) INDIVIDUAL ISOLATE: Swiss-Prot P33567

25 (vii) IMMEDIATE SOURCE:
 (B) CLONE: alpha synuclein protein

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Asp Val Phe Met Lys Gly Leu Ser Met Ala Lys Glu Gly Val Val
 1 5 10 15

35 Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Thr Glu Ala Ala Glu Lys
 20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45

40 Val Gln Gly Val Ala Ser Val Ala Glu Lys Thr Lys Glu Gln Ala Ser
 50 55 60

45 His Leu Gly Gly Ala Val Phe Ser Gly Ala Gly Asn Ile Ala Ala Ala
 65 70 75 80

Thr Gly Leu Val Lys Lys Glu Glu Phe Pro Thr Asp Leu Lys Pro Glu
 85 90 95

50 Glu Val Ala Gln Glu Ala Ala Glu Glu Pro Leu Ile Glu Pro Leu Met
 100 105 110

Glu Pro Glu Gly Glu Ser Tyr Glu Glu Gln Pro Gln Glu Glu Tyr Gln
 115 120 125



Glu Tyr Glu Pro Glu Ala
130

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 142 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Serinus canaria*
(C) INDIVIDUAL ISOLATE: genbank L33860

(vii) IMMEDIATE SOURCE:

- (B) CLONE: alpha synuclein homologue

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met	Asp	Val	Phe	Met	Lys	Gly	Leu	Ser	Lys	Ala	Lys	Glu	Val	Val	Ala	1	5	10	15
Ala	Ala	Glu	Lys	Thr	Lys	Gln	Gly	Val	Ala	Glu	Ala	Ala	Gly	Lys	Thr	20	25	30	
Lys	Glu	Gly	Val	Leu	Tyr	Val	Gly	Ser	Arg	Thr	Lys	Glu	Gly	Val	Val	35	40	45	
His	Gly	Val	Thr	Thr	Val	Ala	Glu	Lys	Thr	Lys	Glu	Gln	Val	Ser	Asn	50	55	60	
Val	Gly	Gly	Ala	Val	Val	Thr	Gly	Val	Thr	Ala	Val	Ala	Gln	Lys	Thr	65	70	75	80
Val	Glu	Gly	Ala	Gly	Asn	Ile	Ala	Ala	Ala	Thr	Gly	Leu	Val	Lys	Lys	85	90	95	
Asp	Gln	Leu	Ala	Lys	Gln	Asn	Glu	Glu	Gly	Phe	Leu	Gln	Glu	Gly	Met	100	105	110	
Val	Asn	Asn	Thr	Gly	Ala	Ala	Val	Asp	Pro	Asp	Asn	Glu	Ala	Tyr	Glu	115	120	125	
Met	Pro	Pro	Glu	Glu	Glu	Tyr	Gln	Asp	Tyr	Glu	Pro	Glu	Ala			130	135	140	



(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 143 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Torpedo californica*
 (C) INDIVIDUAL ISOLATE: Swiss-Prot P37379

(vii) IMMEDIATE SOURCE:

- (B) CLONE: alpha synuclein homologue

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Met Asp Val Leu Lys Lys Gly Phe Ser Phe Ala Lys Glu Gly Val Val
1           5           10           15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Gln Asp Ala Ala Glu Lys
20           25           30

Thr Lys Gln Gly Val Gln Asp Ala Ala Glu Lys Thr Lys Glu Gly Val
35           40           45

Met Tyr Val Gly Thr Lys Thr Lys Glu Gly Val Val Gln Ser Val Asn
50           55           60

Thr Val Thr Glu Lys Thr Lys Glu Gln Ala Asn Val Val Gly Gly Ala
65           70           75           80

Val Val Ala Gly Val Asn Thr Val Ala Ser Lys Thr Val Glu Gly Val
85           90           95

Glu Asn Val Ala Ala Ala Ser Gly Val Val Lys Leu Asp Glu His Gly
100          105          110

Arg Glu Ile Pro Ala Glu Gln Val Ala Glu Gly Lys Gln Thr Thr Gln
115          120          125

Glu Pro Leu Val Glu Ala Thr Glu Ala Thr Glu Glu Thr Gly Lys
130          135          140

```

(2) INFORMATION FOR SEQ ID NO:9:



4

5

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7

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
5 (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "primer #1F"
10 (iii) HYPOTHETICAL: NO

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACGACAGTGT GTGTAAAGG

19

20 (2) INFORMATION FOR SEQ ID NO:10:

 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
25 (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "primer #13R"
30 (iii) HYPOTHETICAL: NO

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AACATCTGTC AGCAGATCTC

20

(2) INFORMATION FOR SEQ ID NO:11

40 (i) SEQUENCE CHARACTERISTICS
 (A) LENGTH: 2809 base pairs
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: DOUBLE
 (D) TOPOLOGY: LINEAR

45 (ii) MOLECULAR TYPE: DNA (genomic)
 (iii) HYPOTHETICAL: NO



(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 139A20 HUMAN BETA SYNULEIN GENE

(vi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

5 CCGCCGCAGC CGCCGCTCCA TCCCCAGCCC CGGCCCCGCA TCCGGTTTGG AAGGGGGCTG
CAAGTTTGCA AGGGGCCCCG GANAAAAANC GAGCAGTGGC CCTTCCCGCG TCCCCAGGGT
TTCAAGGGAC GCTAGGANTN TCCGCGGCCC TGGAGGTTCT CACTGGGGAG TGGGGTGAGA
TGGGGGGAAA GCGGGAGGGG GCTCAGGGTC CAGAAGGGCN CCGCGGTCTC GGGAGTAGGG
GGGCATNTGC GTCCCGCGGG AGGGGCTGGG GTGAGAGTGC GGGGCCAGTG CACCGGTGCC
10 CGTGTATCGC CCTCCCCAGG CCGCCAGGAT GGACGTGTTC ATGAAGGGCC TGTCCATGGC
CAAGGAGGGC GTTGTGGCAG CCGCGGAGAA AACCAAGCAG GGGGTCACCG AGGCGGCGGA
GAAGACCAAG GAGGGCGTCC TCTACGTCGG TGGGCNNGGG GCNNGGTTTC TGGGGCTGCA
GGGCTGGGGG TCCCCCTACA GTGTGGAGCT GGGGCCGGGT CCCGGGGAGG GGGGTTCTGG
GCAAGATAAT ATNANTCAGC AGATGGGGCN AGGTCANCAN GGGTCATAAG GGACATACCC
15 ANCCCATAGA ANCCTGGGTC TGTATCCGGA AATGGGGACA CGGGGCGGGC TGATGAGGTG
GGGGGCTCCA NCTGAAAGGC CAGGGACCAN TGCANTNATA AAANCACACA NCCTCCTTTT
TCTTATCTTT TTTACCATTA TTAATAGTTA TCTGGTGTTG AACACTTTCT GTATGCCAAG
TACTGGGTAA AATGTCATAA CATCCATTTC CTCATGTAAT GCTTCCGCCC ATTCTACAGG
TAAGGGAAAC TGGGCTTCCC ATTGGTAGNT AAATTTTAGG TTCAGAAAGG CTTGAATTGA
20 ATGTCAGTTC AGCCAATTTT TTAGTGGTGG AACCAAAGT AGTTCCATCC GTGAAACGGG
GACAATAACA GCACCCGCTT CCCAGGGCTG GGGAAAAGTG AAGTGCAGCG GGGCAGGCAG
AGGACTTGAC ACAGCACTGG CCCTCAGCCA ACATCCACTA GAGGGGTGGG GTATCGCATC
AGGTGGGAGA GAACTGCAAC CCTTGCAGAC AGAGGTGTGG GGCCAGTGTC AGTGATAAGA
CGGGGGTTAA CATGGGGGTG CAGGTTGTAG GATNTGGGGA CCCAAGGAGG CAGTGACGGG
25 GCCAGGATGC CCACTCTGTA ATCACCATGC TGTGCTGGAG TTTCTGTTCC CTCAGCGCAG
AGTCCTTAAA TGTGCCGCTT TTTCTNCCCT GCAGGAAGCA AGACCCGAGA AGGTGTGGTA
CAAGGTGTGG CTTCAGGTAC TAGCCCAGCC CTGGCACCAG CCCTTCTCTC AMTTAGGCGG



ATGATCTGGC CGGGAACCAG AGGGCGGGG CGGGGAGAC TCCCAAGGCT TCTGCGGGAA
TGCTCCGTGG GGAGGGCAGG CCCTGGGATA CTACAAGGCA GGGCATCGGT GTTCCCCCT
GGCTCCCAA CCCCTTCCTC AACCCCCTCC CTGCTCCAGT GGCTGAAAA ACCAAGGAAC
AGGCCTCACA TCTGGGAGGA GCTGTGTTCT CTGGGGCAGG GAACATCGCA GCAGCCACAG
5 GACTGGTGAA GAGGGAGGAA TTCCCTACTG ATCTGAAGGT AAGCGATCCT TCTGACCCGC
ACATGCAGGC AAACACACAC ACACACACAC ACACACACCN GGCACACAAA TAAACCTGTC
ACCATCCCCG CCCCCCTAAT CCTGCCACCA GCTTGGAACA CAAGCCACTT TGCCTCCCAT
CCTGCNGGCC CGTGCTAGAC TCAGCTCAGA ATGCATCTGA ATAANGGCGT GCATGGGTGT
GACGCTCCCG GTGATGGGGA CCCAGACCTG GCTGTCTGCG TGTATCCTGC TTGCCAGCGT
10 GACCCATATG ACTTCTGGCC ACGTCTGCAT GTGTCAATGA TTGTTTATTC ATTTCTTTTC
ATTCAACAAA TATCCATGCC ANANCCAGCC CTGTCCTTGA GCTTCCAGNT CCCTTTCAGC
CNAGGGGAGC NTGAGGGTTA TTTTGGGGT CCCGATGCCC AGCACAGAGC CTGACACAAA
GGATGAGGCA TAAGCTGGTG ANTGAGTATC CAAATGGTGG AAGTGTGGAG GNTGCCAGGC
ATTGGGGGAG CGGCGTGGAG AGCCAGCTCC CCAATCCATG CTGCCACTTC AACTGTGATT
15 CGGGGGAATT TCCCCCTTCA CCTCCATCCC ACTTCCAAGG CACTCCAAAT AAATAACTGA
ATTAGAAATT ATCCTTGTTT TGCCAACCCA CCCTAGCCTT CCCCCTCCA ACCCACCCTA
AGCTTACCAC TGTGGGAATT TGGGGGGCAT CCTGGCTGTC CTCACGAGTC CTGACCTTTT
CTGCCCACAG CCAGAGGAAG TGGCCCAGGA AGCTGCTGAA GAACCACTGA TTGAGCCCCT
GATGGAGCCA GAAGGGGAGA GTTATGAGGA CCCACCCAG GAGGAATATC AGGAGTATGA
20 GCCAGAGGCG TAGGGGCCCA GGAGAGCCCC CACCAGCAGC ACAATTCTGT CCCTGTCCCT
GCCCCGCCCC CCAGAGCCAG GGCTGTCCTT AGACTCCTTC TCCCCAATCA CGAGATCTTC
CTTCCGCTCT GAGGCAACCC CCTCGGAGCC TGTGTTAGTG TCTGTCCATC TGTCTGTCCT
ACCCGCCCCG GTCCAACCCC GGGGCATGGA CAGGGCCAGG GTTGCGGTCTG CGGCTGGGAG
CCTCGCCCCT CAGTGTGTC CTCCTCCCAT CCAGCGTCTG CGCG

25

(2) INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISTICS



(A) LENGTH: 223 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

5 (ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 174P13 HUMAN GAMMA SYNULEIN GENE, 5' END

10 (vi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGGGAGATCC AGCTCCGTCC TGCCTGCAGC AGCACAACCC TGCACACCCA CCATGGATGT
CTTCAAGAAG GGCTTCTCCA TCGCCAAGGA GGGNGTGGTG GGTGCGGTGG AAAAGACCAA
GCAGGGGGTG ACGGAAGCAG CTGAGAAGAC CAAGGAGGGG GTCATGTATG TGGGATTACA
TTTTTTTTTTT AAAGAAAGAA TAAATTAATT GTGATTAAAG TTG

15

(2) INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 677

(B) TYPE: NUCLEIC ACID

20 (C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

25 (v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 174P13 HUMAN GAMMA SYNULEIN GENE, 3' END

(vi) SEQUENCE DESCRIPTION: SEQ ID NO:13:



TTTTTTNAGG GGGGAAAACA GGGAATANAA AAANANGGGG GGGGGTTTTT NNGGGGGGGG
GGGGAAAANG GTTNGGGGGN NAACCNAAN AAANNCCNAN GGGGGGGGNN ANTNAANTTT
TGGGAACCCA AAGCCCNAGG AGGATTTTTN GTNAANAACG TNACCTCNAG TGGGNCGAGG
AAGACCAAGG AAANGCCCAA CNCGTTGAN CGAGGCTGTG GTGAACANCG TNCAACNCTG
5 TGCCCNCCAA NANC GTGAG GNGGCGGAGA ACATCSCGGT CACCTCCGGG GTGGTGCGCM
AGGAGGACTT GAGGCCATCT KCCCCCMAC AGGAGGGTGT GGCATCCMAA GARAAAGAGG
AAGTGGCAGA GGAGGCCAG AGTGGGGGAR ACTAGAGGGC TACAGGCCAG CGTGGATGAC
CTGAAGAGCG CTCCTCTGCC TTGGACACCA TCCCCTCCTA GCACAAGGAG TGCCCGCCTT
GAGTGACATG CGGCTGCCCA CGCTCCTGCC CTCGTCTTCC TGGCCACCCT TGGCCTGTCC
10 ACCTGTGCTG CTGCACCAAC CTCACTGCCC TCCCTCGGCC CCACCCACCC TCTGTCCTT
CTGACCCAC TTATGCTGCT GTGAATTTTT TTTTAAATG ATTCAAATA AACTTGAGC
CCACTCCAAA AAAAAAA

15 (2) INFORMATION FOR SEQ ID NO:14

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1181 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

20 (D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

25 (A) CLONE: human alpha synuclein gene/ exons 1 and 2 plus
flanking intron sequences

(viii) POSITION IN GENOME:



(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
AATTTTCAGCG ATGCGAGGGC AAAGCGCTCT CGGCGGTGCG GTGTGAGCCA CCTCCCGGCG
5 CTGCCTGTCT CCTCCAGCAG CTCCCCAAGG GATAGGCTCT GCCCTTGGTG GTCGACCCTC
AGGCCCTCGN TCTCCCAGGN CGACTCTGAC GAGGGGTAGG GGGTGGTCCC CNGGAGGACC
CAGAGGAAAG GCNNGGACAA GAAGGGAGGG GAAGGGGAAA GAGGAAGAGG CATCATCCCT
AGCCCAACCG CTCCCGATCT CCACAAGAGT GCTCGTGACC CTAAACTTAA CGTGAGGCGC
AAAAGCGCCC CAACCTTTTC CCGCCTTGNN CCAGGCAGGC GGCTGGAGTT GATGGCTCAC
10 CCCGCGCCCC CTGCCCCATC CCCATCCGAG ATAGGGACGA GGAGCACGCT GCAGGGAAAG
CAGCGAGCCG CGGGAGAGGG GCGGGCAGAA GCGCTGACAA ATCAGCGGTG GGGGCGGAGA
GCGGAGGAGA AGGAGAAGGA GGAGGACTAG GAGGAGGAGG ACGGCGACGA CCAGAAGGGG
CCCAAGAGAG GGGGCGAGCG ACCGAGCGCC GCGACGCGAA GTGAGGTGCG TCGGGGCTCA
GCGCAGACCC CGGCCCGGCC CCTCCTGAGA GCGTCCTGGG CGCTCCCTCA CGCCTTGCCT
15 TCAAGCCTTC TGCCTTTCCA CCCTCGTGAG CGGAGAACTG GGAGTGGCCA TTCGACGACA
GGTTAGCGGG TTTGCCTCCC ACTCCCCCAG CCTCGCGTCG CCGGCTCACA GCGGCCTCCT
CTGGGGACAG TCCCCCCCCG GTGCCCCTCC GCCCTTCCTG TCGCTCCTT TTCCTTCTC
TTTCCTATTA AATATTATTT GGGAATTGTT TAAATTTTTT TTTTAAAAA AGAGAGAGGC
GNGGAGGAGT CGGAGTTGTG GAGAAGCAGA GGGACTCAGG TAAGTACCTG TGGATCTAAA
20 CGGGNGTCTT TTGGAAATCC TGGAGAACGC CGGATGGAGA CGAATGGTCG TGGGNACCGG
GAGGGGGTGG TGCTGCCATG AGGACCGCTG GGCCAGGTCT CTGGGAGGTG AGTACTTGTC
CTTTGGGGAG CTAAGGAAAG AGACTTGACC TGGCTTTCGT CCTGCTTCTG ATATTCCCTT
CTCCACAAGG GCTGAGAGNT TAGGCTGCTT CTCCGGGATC C
```

25 (2) INFORMATION FOR SEQ ID NO:15

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 536 base pairs



(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

5 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exon 3 plus flanking
intron sequences

10 (viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTTAAAAGAG TCTCACA CTT TGGAGGGTTT CTCATGATTT TTCAGTGTTT TTTGTTTATT
15 TTTCCCCGAA AGTTCTCATT CAAAGTGTAT TTTATGTTTT CCAGTGTGGT GTAAAGAAAT
TCATTAGCCA TGGATGTATT CATGAAAGGA CTTTCAAAGG CCAAGGAGGG AGTTGTGGCT
GCTGCTGAGA AAACCAAACA GGGTGTGGCA CAAGCAGCAG GAAAGACAAA AGAGGGTGTT
CTCTATGTAG GTAGGTAAAC CCCAAATGTC AGTTTGGTGC TTGTTTCATGA GTGATGGGTT
AGGATAACAA TACTCTAAAT GCTGGTAGTT CTCTCTCTTG ATTCATTTTT GCATCATTGC
20 TTGTCAAAAA GGTGGACTGA GTCAGAGGTA TGTGTAGGTA GGTGAATGTG AACGTGTGTA
TNTGAGCTAA TAGTAAAAAT GCGACTGTTT GCTTTTCAGA TTTTAAATTT TGCCTAATAT
NTATGACTTN TTAAAATGAA TGTTTCTGTA CTACATAATT CTATNTCAGA GACAGT

(2) INFORMATION FOR SEQ ID NO:16

25 (i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 650 base pairs

(B) TYPE: NUCLEIC ACID



(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

5 (iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exon 4 plus flanking
intron sequences

(viii) POSITION IN GENOME:

10 (A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGCAGGTCA ACGGATCTGT CTCTAGTGCT GTACTTTTAA AGCTTCTACA GTTCTGAATT
CAAAATTATC TTCTCACTGG GCCCCGGTGT TATCTCATTC TTTTCTCC TCTGTAAGTT
15 GACATGTGAT GTGGGAACAA AGGGGATAAA GTCATTATTT TGTGCTAAAA TCGTAATTGG
AGAGGACCTC CTGTTAGCTG GGCTTTCTTC TATNTATTGT GGTGGTTAGG AGTTCCTTCT
TCTAGTTTTA GGATATATAT ATATATTTTT TCTTCCCTG AAGATATAAT AATATATATA
CTTCTGAAGA TTGAGATTTT TAAATTAGTT GTATTGAAAA CTAGCTAATC AGCAATTTAA
GGCTAGCTTG AGACTTATGT CTTGAATTTG TTTTGTAGG CTCCAAAACC AAGGAGGGAG
20 TGGTGCATGG TGTGGCAACA GGTAAGCTCC ATTGTGCTTA TATCAAAGAT GATATNTAAA
GTATCTAGTG ATTAGTGTGG CCCAGTATCA AGATTCCTAT TGAAATTGTA AAACAATCAC
TGAGCATCTA AGAACATATC AGTCTTATTG AACTGAATT CTTTATAAAG TATTTTAA
TAGGTAAATA TTGATTATAA ATAAAAATA TACTTGCCAA GAATAATGAG

25 (2) INFORMATION FOR SEQ ID NO:17

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 504 base pairs



(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

5 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exon 5 plus flanking
intron sequences

10 (viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATATCTTAGC CAAGATTCAA TGTTTGTTG AACCACTC ACTTGACATC TTGGTGGCTT
15 TTGTTTCTTC TGACCACTCA GTTATCTATG GCATGTGTAG ATACAGGTGT ATGGAANCGA
TGGCTAGTGG AAGTGGAATG ATTTAAGTC ACTGTTATTC TACCACCCTT TAATCTGTTG
TTGCTCTTTA TTTGTACCAG TGGCTGAGAA GACCAAAGAG CAAGTGACAA ATGTTGGAGG
AGCAGTGGTG ACGGGTGTGA CAGCAGTAGC CCAGAAGACA GTGGAGGGAG CAGGGAGCAT
TGCAGCAGCC ACTGGCTTTG TCAAAAAGGA CCAGTTGGGC AAGGTATGGC TGTGTACGTT
20 TTGTGTTACA TTTATAAGCT GGTGAGATTA CGGTTCAATT TCATGTGAAG CCTGGAGGCA
GGAGCAAGAT ACTTACTGTG GGGAACGGCT ACCTGACCCT CCCCTTGTGA AAAAGTGCTA
CCTTTATATT GGTCTTGCTT GTTT

(2) INFORMATION FOR SEQ ID NO:18

25 (i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 727 base pairs

(B) TYPE: NUCLEIC ACID



(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

5 (iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exons 1 and 2 plus
flanking intron sequences

(viii) POSITION IN GENOME:

10 (A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AAAAGTTTAC ATACTTTGAG GTTGATAACC CATGTTGCCG CAATGTTTCC CCGGAGGCAT
TGTGGAGTTT AGAATGCCAG TAGTAATATT AAGGTGTGCC ATTTTCAAGA TCCGTGGCCA
15 ACATCCCTAT ATGTAAGATT TTTCCAAAAC ATGTTTCTGA TTTTAAAAG TGAAAAATGC
TACTTCATCA TGTTCTTTTT GTGCTTCTTA CTTTAAATAT TAGAATGAAG AAGGAGCCCC
ACAGGAAGGA ATTCTGGAAG ATATGCCTGT GGATCCTGAC AATGAGGCTT ATGAAATGCC
TTCTGAGGTA GGAGTCCAAG CTGAATCTTT CTAACAAGAC AGTACCAAAA ACCTGTCATT
GTCACATTTT TCTTTCATTA GTGCTTAGTG AGAATCATTT GCTCTCTACA TGCTCATTA
20 GTGGACAAC TGAAGTTAA GAATAGTTTT TACATTTTTT AAGGGTCCTT AAAAAAAAAG
AGGAGGAGGA AGATGAAGAA GAGGAAGAAA GGATGTAAAA GAAATCATAT GTAGTCCACA
TAGCTTAATA TACNTACTAC TTGACCCTTT ACAGGAAAAG CTTTACTAAC CCCTGCATTA
GAGAATATAT TTTTTTGCAA AAACATTGAT TGTAATTTT AGTGTAAGT GGGGAGCCAT
TTCCTATCTC ATTGGCTGTC CAGTGCTGAT GCGTAATTGA AACTTATACT AACAGTGTGT
25 GCTGTCT

(2) INFORMATION FOR SEQ ID NO:19



(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1596 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

5 (D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

10 (A) CLONE: human alpha synuclein gene/ exon 7 plus flanking
intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTTTGATTTT TCTAATATTA GGAAGGGTAT CAAGACTACG AACCTGAAGC CTAAGAAATA
TCTTTGCTCC CAGTTTCTTG AGATCTGCTG ACAGATGTTC CATCCTGTAC AAGTGCTCAG
TTCCAATGTG CCCAATCATG ACATTTCTCA AAGTTTTTAC AGTGATCTC GAAGTCTTCC
ATCAGCAGTG ATTGAAGCAT CTGTACCTGC CCCCCTCAG CATTTCCGGTG CTTCCCTTTC
20 ACTGAAGTGA ATACATGGTA GCAGGGTCTT TGTGTGCTGT GGATTTTGTG GCTTCAATCT
ACGATGTAA AACAAATTAA AAACACCTAA GTGACTACCA CTTATTTCTA AATCCTCACT
ATTTTTTTGT TGCTGTTGTT CAGAAGTTGT TAGTGATTTG CTATCATATA TTATNAGATT
TTTAGGTGTC TTTTAATGAT ACTGTCTAAG AATAATGACG TATTGTGAAA TTTGTTAATA
TATATNATAC TTAATAATAT GTGAGCATGA AACTATGCAC CTATAATACT AAATATGAAA
25 TTTTACCATT TTGCGATGTG TTTTATTCAC TTGTGTTTGT ATATNAATGG TGAGAATTAA
AATAAACGT TATCTCATTG CAAAAATATT TTATTTTAT CCCATCTCAC TTTAATAATA
AAAATCATGC TTATAAGCAA CATGAATTAA GAACTGACAC AAAGGACAAA AATATAAAGT



TATTAATAGC CATTGAAGA AGGAGGAATT TTAGAAGAGG TAGAGAAAAT GGAACATTAA
CCCTACACTC GGAATTCCTT GAAGCAACAC TGCCAGAAAGT GTGTTTTGGT ATGCACTGGT
TCCTTAAGTG GCTGTGATTA ATTATTGAAA GTGGGGTGTT GAAGACCCCA ACTACTATTG
TAGAGTGGTC TATTTCTCCC TTCAATCCTG TCAATGTTTG CTTTACGTAT TTTGGGGAAC
5 TGTGTTTTGA TGTGTATGTG TTTATAATTG TTATACATTT TTAATTGAGC CTTTTATTAA
CATATATTGT TATTTTTGTC TCGAAATAAT TTTTGTAGTA AAATCTATTT TGTCTGATAT
TGGTGTGAAT GCTGTACCTT TCTGACAATA AATAATATNC GACCATGAAT AAAAAAAAAA
AAAAAGTGGG TTCCCGGGAA CTAAGCAGTG TAGAAGATGA TTTTGACTAC ACCCTCCTTA
GAGAGCCATA AGACACATTA GCACATATTA GCACATTCAA GGCTCTGAGA GAATGTGGTT
10 AACTTTGTTT AACTCAGCAT TCCTCACTTT TTTTTTTTAA TCATCAGAAA TTCTCTCTCT
CTCTCTCTTT TTCTCTCGCT CTCTTTTTTT TTTTTTTTTT TTTTACAGGA AATGCCTTTA
AACATCGTTG GGAACACCA GAGTCACCTT AAAGGGAGNA TCAATTCTCT AGGACTGGAT
AAAAATTTCA TGGGCCTCCT TTAAAATGTT GCCCAAATAT ATGGAATTCT AGGGGTTTTT
CCNTAGGGGG AAGGGTTTTT TCTCTTTTCN GGGGAGGATC CTTTAAACNC CCCNGGGGGG
15 NGCCCGGAAA ATAAACTTGG NGGGGGGGNA AACTT

20



WHAT IS CLAIMED IS:

1. An isolated nucleic acid comprising a nucleotide sequence encoding a mutated human synuclein protein or homologue thereof.

25 2. The isolated nucleic acid of claim 1 wherein said mutated synuclein protein is selected from the group consisting of alpha, beta and gamma synuclein proteins.

30 3. The isolated nucleic acid of claim 2 wherein said mutated synuclein protein is the alpha synuclein protein.

4. The isolated nucleic acid of claim 3 wherein said nucleotide sequence contains at least one mutation at base pair position 209.

35 5. The isolated nucleic acid of claim 4 wherein said mutation at position 209 is a change from guanine to adenine.

6. The isolated nucleic acid of claim 5 having the sequence given in SEQ ID NO. 1.

40

7. An oligonucleotide complementary to a portion of the synuclein gene, wherein said portion comprises a mutation associated with predisposition to Parkinson's Disease.

45 8. The oligonucleotide of claim 7 wherein said mutation is at base pair position 209 in the synnuclein gene.



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4

9. The oligonucleotide of claim 8 wherein said mutation is a change from guanine to adenine.

10. A vector comprising the isolated nucleic acid of claim 1.

5

11. A host cell comprising the vector of claim 10.

12. A method of affecting characteristics of Parkinson's Disease, comprising expressing nucleic acids which are implicated in disease development in cultured cells through the use of expression vectors.

10

13. The method of claim 12 wherein the said nucleic acid is selected from the group consisting of alpha, beta, and gamma synuclein genes.

14. The method of claim 13 wherein the said nucleic acid encodes the mutated alpha synuclein protein.

15

15. The method in claim 14 wherein the said mutated alpha synuclein protein contains at least one mutation at base pair 209.

20

16. The method of claim 15 wherein said mutation at position 209 is a change from guanine to adenine.

17. An isolated human synuclein protein or peptide containing at least one mutation.

25

18. The isolated human synuclein protein or peptide of claim 17 wherein said protein or peptide is selected from the group consisting of



1

4

the human alpha, beta and gamma synuclein proteins or fragments thereof.

19. The isolated human synuclein protein or peptide of claim 18 having the sequence given in SEQ ID NO 5.

5

20. The isolated human synuclein protein or peptide of claim 19 wherein said protein or peptide is the alpha synuclein gene or a fragment thereof.

10 21. The isolated protein or peptide of claim 20, wherein said mutation is at amino acid position 53.

22. The isolated protein or peptide of claim 21, wherein said mutation is an alanine to threonine substitution.

15

23. An antibody specific for the protein or peptide of claim 17.

24. A method of detecting subjects at increased risk for Parkinson's Disease, comprising:

20 obtaining a sample comprising nucleic acids, proteins or tissues from the subjects, and

detecting in the nucleic acids, proteins or tissues the presence of a mutation which is associated with Parkinson's disease,

thereby identifying subjects at increased risk for the disease.

25

25. The method of claim 24 wherein said mutation is located on human chromosome four.



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26. The method of claim 25 wherein said mutation is located in the alpha synuclein gene.

27. The method of claim 26 wherein said mutation causes an amino
5 acid substitution at position 53.

28. The method of claim 27 wherein said mutation causes an alanine to threonine substitution at position 53.

10 29. The method of claim 24 wherein said detecting step comprises combining a nucleotide probe which selectively hybridizes to a nucleic acid containing said mutation, and detecting the presence of hybridization.

15 30. The method of claim 29 wherein said nucleotide probe is an oligonucleotide complementary to a portion of the synuclein gene, wherein said portion comprises a mutation associated with predisposition to Parkinson's Disease.

20 31. The method of claim 30 wherein the mutation of said oligonucleotide is at base pair position 209 in the alpha synuclein gene.

32. The method of claim 31 wherein the mutation of said oligonucleotide is a change from guanine to adenine.

25

33. The method of claim 24 wherein said detecting step comprises amplifying a nucleic acid product comprising said mutation, and detecting



the presence of said mutation in the amplified product.

34. The method of claim 33 wherein said detecting step comprises selectively amplifying a nucleic acid product comprising said mutation,
5 and detecting the presence of amplification.

35. The method of claim 34 wherein said amplifying step comprises at least one annealing step whereby at least one oligonucleotide is annealed to said sample of nucleic acids.

10

36. The method of claim 35 wherein said amplifying step uses two oligonucleotides.

37. The method of claim 36 wherein said two oligonucleotides have
15 the sequences of SEQ ID NOs 2 and 3.

38. The method of claim 24 wherein said detecting step comprises detecting the presence or absence of a restriction endonuclease site as detected by enzymatic digest of said sample of nucleic acids.

20

39. The method of claim 38 wherein said restriction endonuclease site is recognized by *Tsp451*.

40. The method of claim 24 wherein said detecting step comprises
25 chain termination with a labeled dideoxynucleotide.

41. An oligonucleotide complementary to a nucleic acid sequence



which flanks a mutation in the synuclein gene that is associated with predisposition to Parkinson's disease, wherein said oligonucleotide may be used in diagnostic screens in the amplification of a nucleic acid sequence comprising said mutation.

5

42. The oligonucleotide of claim 41 having the sequence of SEQ ID NO 2.

43. The oligonucleotide of claim 41 having the sequence of SEQ ID
10 NO 3.

44. The method of claim 24 wherein said detection step comprises identification of said mutations with an antibody.

15 45. The method of claim 44 wherein said antibody is directed against an isolated human synuclein protein or peptide containing at least one mutation.

46. The method of claim 45 wherein said isolated human synuclein
20 protein or peptide is selected from a group consisting of the human alpha, beta, and gamma synuclein proteins or fragments thereof.

47. The method of claim 46 wherein said isolated human synuclein protein or peptide has the mutated sequence given in SEQ ID NO 5.

25

48. The method of claim 47 wherein said mutation is at amino acid position 53.



49. The method of claim 48 wherein said mutation is an alanine to threonine substitution

50. A diagnostic kit comprising the oligonucleotide of claim 41.

5

51. A diagnostic kit comprising the oligonucleotide of claim 42.

52. A diagnostic kit comprising the oligonucleotide of claim 43.

10

53. A diagnostic kit comprising the oligonucleotide of claim 7.

54. A diagnostic kit comprising the oligonucleotide of claim 8.

55. A diagnostic kit comprising the oligonucleotide of claim 9.

15

56. A diagnostic kit comprising the antibody of claim 23.

57. An isolated nucleic acid comprising a mutation in a human synuclein gene or homologue thereof.

20

58. The isolated nucleic acid of claim 57 wherein said synuclein gene is the alpha synuclein gene.

59. The isolated nucleic acid of claim 58 wherein said mutation occurs at base pair position 209.

25

60. The isolated nucleic acid of claim 59 wherein said mutation is



a change from guanine to adenine.

61. The isolated nucleic acid of claim 60 having the sequence given in SEQ ID NO 1.

5

62. A transgenic animal which expresses a mutated synuclein protein, wherein said animal may be used as an animal model for Parkinson's disease.

10

63. The transgenic animal of claim 62, wherein said mutated synuclein protein is an alpha synuclein protein.

15

64. A method of screening a compound for the ability to reverse the self-aggregation of synuclein proteins, comprising exposing an aggregate of synuclein proteins to a test compound and observing whether or not the aggregate is dissolved.

20

65. The method of claim 64 wherein said test compound is a synuclein peptide.

66. The method of claim 65 wherein said peptide comprises a mutation.

25

67. The method of claim 64 wherein said test compound is an antibody.

68. The method of claim 64, wherein said observing step comprises



Congo red staining, electron microscopy or CD spectrometry.

69. The method of claim 64 wherein said protein aggregate is located within an animal.

5

70. A method of screening a compound for the ability to inhibit the self-aggregation of synuclein proteins, comprising exposing a population of synuclein proteins to a test compound under conditions which promote self-aggregation in the absence of said compound and observing whether or
10 not self-aggregation of said proteins is inhibited.

71. The method of claim 70 wherein said test compound is a synuclein peptide.

15 72. The method of claim 71 wherein said peptide comprises a mutation.

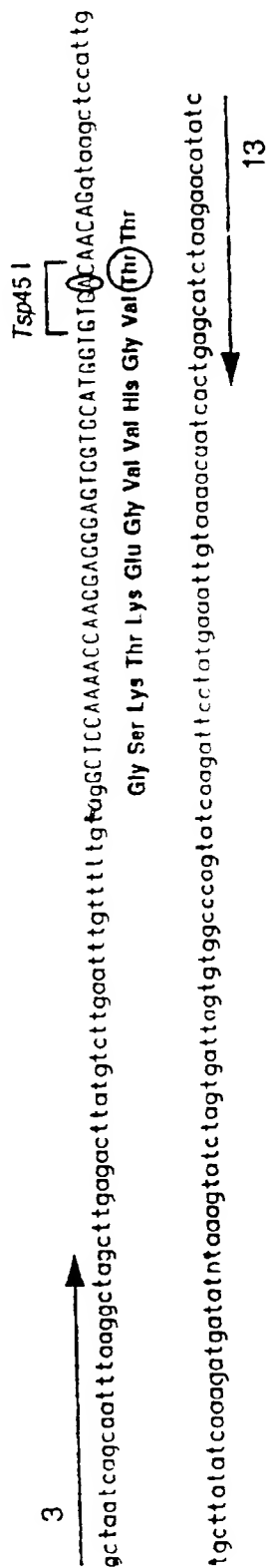
73. The method of claim 70 wherein said test compound is an antibody.

20

74. The invention substantially as disclosed and described.



Figure 1





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Figure 2



Figure 3

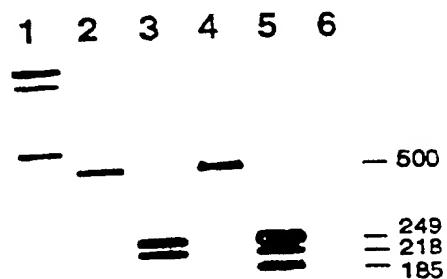
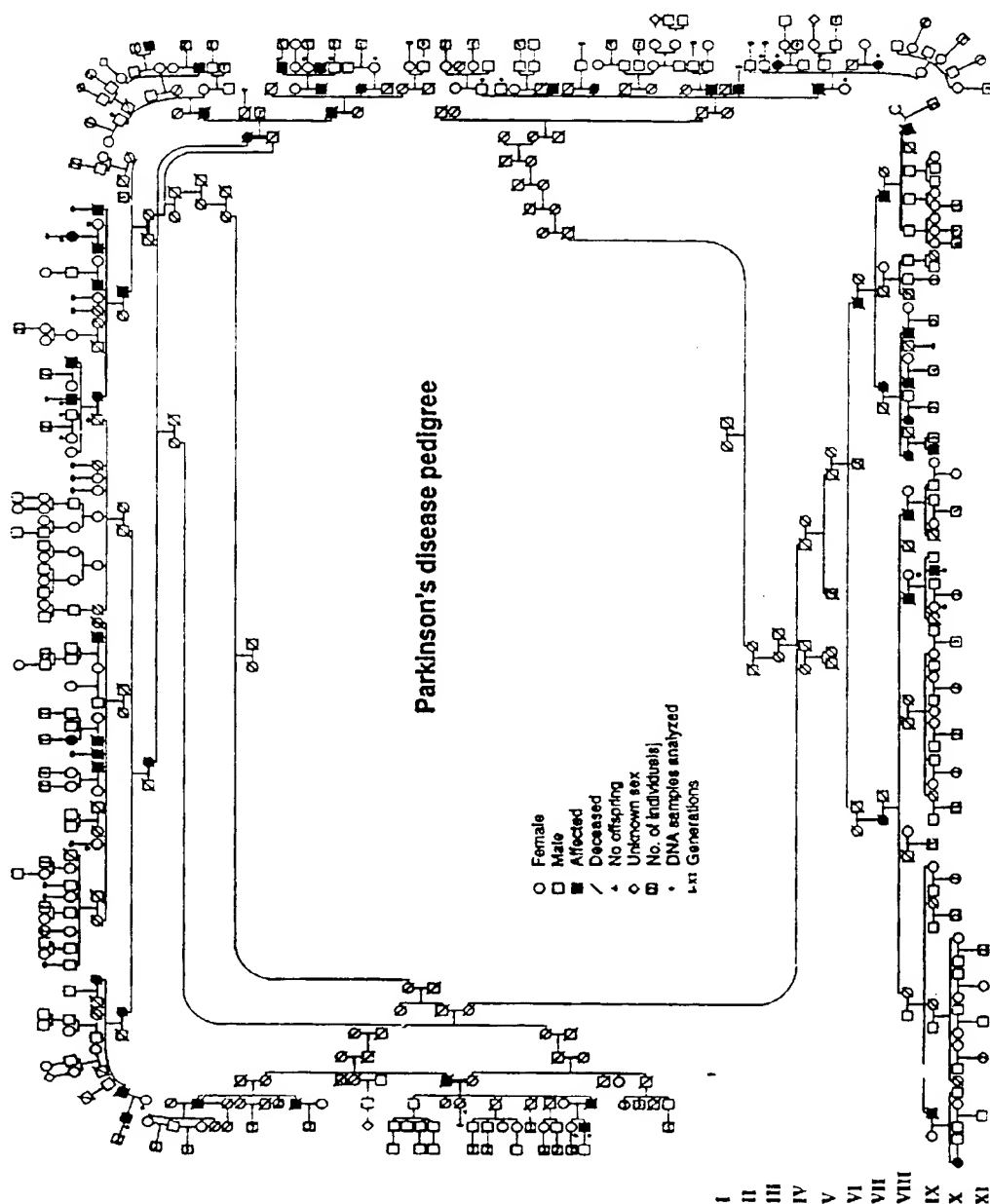






Figure 5





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Figure 6

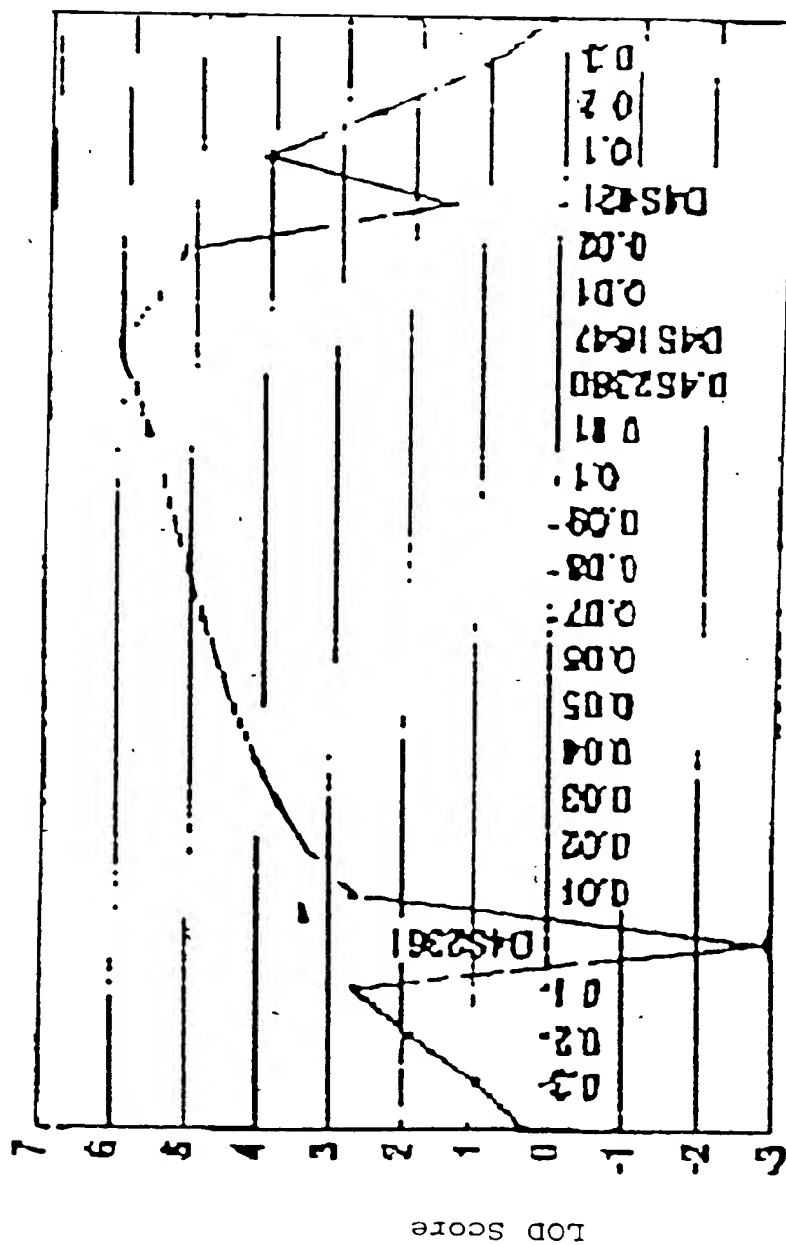




Figure 7

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clone	5'	3'	gene
109979	T84229	T88834	alpha
111088	T83410		alpha
111090	T83411	T81593	alpha
130048	R11819	(R19409)	alpha
135534	R31354	R32856	alpha
141248	R66863	R67383	alpha
145594	R78091	R77746	alpha
171906	H19290	H19291	beta
172284	H19556	H19474	beta
172749		H19685	beta
175546		H41126	beta
193174	H47503	H47504	alpha
210768	H66914	H66869	alpha
213616	H70324	H70325	alpha
236027	H62070		alpha
248153	N53829	N73325	alpha
24991	(T80528)	R39000	alpha
26298	R13508	(R20629)	alpha
265817	N28661	N21457	alpha
266628		N22757	alpha
27342		R37173	alpha
280344	(N50305)	N47094	alpha
290894		N72005	alpha
294142		N68597	alpha
307787	W21278		alpha
340835	W56712	W56757	alpha
340883	W55986	W56276	alpha
346647	W94390	W74638	alpha
346796	W79685	W79784	alpha
359349	AA010546	AA010547	alpha
364632	AA022809	AA022690	alpha
39915		R50455	beta
40764	R56327	R56245	alpha
45086	H06908	H08824	alpha
48607	H10267	H10213	alpha
49811	H29080	H28976	alpha
50202		H17962	beta
50470		H16811	beta
66473	R16018	R16119	alpha
667794	AA258686	AA258608	alpha
69907	T48654	T48655	alpha
72391	AA394097	AA293803	gamma
739009	AA421586		beta
739014	(AA42185)	AA421567	beta
771303		AA443638	gamma
2-4		L36675	alpha
2-5		L36674	alpha
c-01f06		F01363	alpha
c-1rb08	F03254	F06981	alpha
c-2td12	F08836	F11169	alpha
c-28f08	F03751	F07521	alpha
cDNA	S69965		beta
EST01420 (HRBAA27)	M78265		gamma
EST19193	AA317129		beta
EST22040	AA319774		alpha



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Figure 7 cont.

EST26845	T28079		beta
EST31489	AA328063		alpha
EST68G11	W22518		gamma
F1-625D	R29481		alpha
GEN-129D09	D81090		beta
hbc590	T11070		alpha
HIBBA65	T08213	T08212	alpha
	HR70E3R	HR70E3F	alpha
HSNACP0		U46896- 46901	alpha
KK1311	N83633		alpha
		D318839	alpha
		L08850	alpha
	T28735		alpha
	Z20502		alpha



Figure 8

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10 20 30 40 50 60 70
CGCGCGCAGCGCGCGCTCCATCCCCAGCCCCGGCCCCGCATCCGGTTTGGAAAGGGGGCTGCAAGTTTGCA 70
AGGGGGCCGGGAXAAAAAXCAGCAGTGGCCCTTCCCGCGTCCCCAGGGTTTCAAGGGACGCTAGGAXTX 140
TCCGCGGCCCTGGAGGTTCGCACTGGGGAGTGGGGTGAGATGGGGGAAAGCGGGAGGGGGCTCAGGGTC 210
CAGAAGGGGCGCGCGGTCTCGGGAGTAGGGGGGCATXTCGCTCCCGCGGGAGGGGCTGGGGTGAGAGTGC 280
GGGGCCAGTGACCGGTGCCCGTGTATCGCCCTCCCCAGGCCGCCAGGATGGACGTGTTTCATGAAGGGCC 350
360 370 380 390 400 410 420
TGTCCATGGCCAAGGAGGGCGTGTGGCAGCCGCGGAGAAAACCAAGCAGGGGGTCACCGAGGCGGCGGA 420
GAAGACCAAGGAGGGCGTCTCTACGTGCGGTGGGCGGGGGCGXGGTTTCTGGGGCTGCAGGGCTGGGGG 490
TCCCCCTACAGTGTGGAGCTGGGGCCGGGTCCCAGGGAGGGGGTCTGGGCAAGATAATATXATCAGC 560
AGATGGGGCXAGGTCAACAGGGTCATAAGGGACATACCCAXCCCATAGAAXCCTGGGTCTGTATCCGGA 630
AATGGGGACACGGGGCGGGCTGTAGAGTGGGGGGTCCAXCTGAAAGGCCAGGGACCAXTGCAXTXATA 700
710 720 730 740 750 760 770
AAAXCACACAXCCTCTTTTTCTIATCTTTTTTACCATTATTAATAGTTATCTGGTGTGAACACTTTCT 770
GTATGCCAAGTACTGGGTAAATGTCTAACATCCATTTCCTCATGTAATGCTTCCGCCATTCTACAGG 840
TAAGGGAAACTGGGCTTCCATTGGTAGXTAAATTTAGGTTTCAGAAAGGCTTGAATTGAATGTCAGTTC 910
AGCCAATTTCTTAGTGGTGAACCAAACAGTGTCCATCCGTGAAACGGGGACAATAACAGCACCCGCTT 980
CCCAGGGCTGGGGAAAAGTGAAGTGCAGCGGGGAGGCAGAGGACTTGACACAGCACTGGCCCTCAGCCA 1050
1060 1070 1080 1090 1100 1110 1120
ACATCCACTAGAGGGGTGGGGTATCGCATCAGGTGGGAGAGAACTGCAACCCTTGACAGACAGAGGTGTGG 1120
GGCCCACTGCAGTGATAAGACGGGGGTTAACATGGGGGTGCAGGTGTAGGATXTGGGGACCCAAGGAGG 1190
CAGTGACGGGGCCAGGATGCCCACTCTGTAATCACCATGCTGTGCTGGAGTTTCTGTTCCCTCAGCGCAG 1260
AGTCTTAATATGTCGGCTTTTCTXCCCIGCAGGAAGCAAGACCCGAGAAGGTGTGGTACAAGGTGTGG 1330
CTTCAGGTACTAGCCAGCCCTGGCACAGCCCTTCTCTCAMTTAGGCGGATGATCTGGCCGGGAACCCAG 1400
1410 1420 1430 1440 1450 1460 1470
AGGGCGGGGGCGGGGGAGACTCCCAAGGCTTCTGCGGGAATGCTCCGTGGGGAGGGCAGGCCCTGGGATA 1470
CTACAAGGCAGGGCATCGGTGTTTCCCCCTGGCTCCCAACCCCTTCTCTCAACCCCTTCCCTGCTCCAGT 1540
GGCTGAAAAAACCAAGGAACAGGCCTCACATCTGGGAGGAGCTGTGTTCTCTGGGGCAGGGAACATCGCA 1610
GCAGCCACAGGACTGGTGAAGAGGGAGGAATTCCTACTGATCTGAAGGTAAGCGATCCTTCTGACCCGC 1680
ACATGCAGGCAAC 1750
1760 1770 1780 1790 1800 1810 1820
CCCCCTAATCCTGCCACCAGCTTGGAAACACAAGCCACTTTGCCCTCCCATCCTGCXGGCCCGTGCTAGAC 1820
TCAGCTCAGAATGCATCTGAATAAXGGCGTGCATGGGTGTGACGCTCCCGGTGATGGGGACCCAGACCTG 1890
GCTGTCTGCGTGTATCTGCTTGGCAGCGTGACCCATATGACTTCTGGCCACGCTGTCATGTGTCAATGA 1960
TTGTTTCATTTCATTTCTTTTTCATTCAACAAATATCCATGCCAXXCCAGCCCTGTCTTGTAGCTTCCAGT 2030
CCCTTTCAGCCXAGGGGAGCXTGAGGGTTATTTTGGGGTCCCGATGCCAGCACAGAGCCTGACACAAA 2100
2110 2120 2130 2140 2150 2160 2170
GGATGAGGCATAAGCTGGTGAGTATCCAAATGGTGGAAAGTGTGGAGGXTGCCAGGCATTGGGGGAG 2170
CGGCGTGGAGAGCCAGCTCCCAATCCATGCTGCCACTTCAACTGTGATTGGGGGAATTTCCCCCTTCA 2240
CCTCCATCCCACTTCCAAGGCACTCCAAATAAATAACTGAATTAGAAATTATCCTTGTTTTGCCAACCCA 2310
CCCTAGCCTTCCCCACTCCAACCCACCCAAAGCTTACCAGTGTGGGAATTTGGGGGGCATCCTGGCTGTC 2380
CTCAGGAGTCTGACCTTTCTGCCACAGCCAGAGGAAGTGGCCAGGAAGTGTGTAAGAACCAGTGA 2450
2460 2470 2480 2490 2500 2510 2520
TTGAGCCCCTGATGGAGCCAGAAGGGGAGAGTTATGAGGACCCACCCAGGAGGAATATCAGGAGTATGA 2520
GCCAGAGGCGTAGGGGGCCAGGAGAGCCCCACCAAGCAGCAATCTGTCCCTGTCCCTGCCCCGCCCC 2590
CCAGAGCCAGGGCTGTCTTAGACTCCTTCTCCCCAATCACAGAGATCTTCTTCCGCTCTGAGGCAACCC 2660
CCTCGGAGCCTGTGTAGTGTCTGTCCATCTGTCTGTCTTACCCGCCCGCTCCAACCCCGGGGCATGGA 2730
CAGGGCCAGGGTTGCGGTGCGGGTGGGAGCCTCGCCCTCCAGTGTGCTCTCCATCCAGCGTCTG 2800
2810 2820 2830 2840 2850 2860 2870
CGCG 2804



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Figure 9

10 20 30 40
AGGGAGATCCAGCTCCGTCCTGCCTGCAGCAGCACAAACCC 40
TGCACACCCACCATGGATGTCTTCAAGAAGGGCTTCTCCA 80
TCGCCAAGGAGGGXGTGGTGGGTGCGGTGGAAAAGACCAA 120
GCAGGGGGTGACGGAAGCAGCTGAGAAGACCAAGGAGGGG 160
GTCATGTATGTGGGATTACATTTTTTTTTTAAAGAAAGAA 200
210 220 230 240
TAAATTAATTGTGATTAAAGTTG 223

Figure 10

10 20 30 40
TTTTTTXAGGGGGGAAAACAGGGAATAXAAAAAXAXGGGG 40
GGGGGTTTTTTXGGGGGGGGGGGAAAAXGGTTXGGGGGX 80
XAACCCXAAAXAAAXXCCXAXGGGGGGGGXXAXTXAAXTTT 120
TGGGAACCCAAAGCCCXAGGAGGATTTTTXGTXAAXAACG 160
TXACCTCXAGTGGGXCGAGGAAGACCAAGGAAAXGCCCAA 200
210 220 230 240
CXCAGTTGAXCGAGGCTGTGGTGAACAXCGTXCAACXCTG 240
TGCCXCXCAAXAXCGTGGAGGXGGCGGAGAACATCSCGGT 280
CACCTCCGGGGTGGTGCAGCMAGGAGGACTTGAGGCCATCT 320
KCCCCCMACAGGAGGGTGTGGCATCCMAAGARAAAGAGG 360
AAGTGGCAGAGGAGGCCAGAGTGGGGGARACTAGAGGGC 400
410 420 430 440
TACAGGCCAGCGTGGATGACCTGAAGAGCGCTCCTCTGCC 440
TTGGACACCATCCCCTCCTAGCACAAAGGAGTGCCCGCCTT 480
GAGTGACATGCGGCTGCCACGCTCCTGCCCTCGTCTTCC 520
TGGCCACCCTTGCCCTGTCCACCTGTGCTGCTGCACCAAC 560
CTCACTGCCCTCCCCTCGGCCCCACCCACCCTCTGGTCCTT 600
610 620 630 640
CTGACCCCACTTATGCTGCTGTGAATTTTTTTTTTAAATG 640
ATTCCAAATAAACTTGAGCCCACTCCAAAAAATAA 677



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Figure 11

alpha-SYN exons 1-2

10 20 30 40
AATTTTCAGCGATGCGAGGGCAAAGCGCTCTCGGCGGTGCG 40
GTGTGAGCCACCTCCCGGCGCTGCCTGTCTCCTCCAGCAG 80
CTCCCAAGGGATAGGCTCTGCCCTTGGTGGTCGACCCTC 120
AGGCCCTCGNTCTCCAGGNCGACTCTGACGAGGGGTAGG 160
GGGTGGTCCCCNNGGAGGACCCAGAGGAAAGGCNNGGACAA 200

210 220 230 240
GAAGGGAGGGGAAGGGGAAAGAGGAAGAGGCATCATCCCT 240
AGCCCAACCGCTCCCGATCTCCACAAGAGTGCTCGTGACC 280
CTAAACTTAACGTGAGGCGCAAAAGCGCCCAACCTTTTC 320
CCGCCCTGNNCCAGGCAGGCGGCTGGAGTTGATGGCTCAC 360
CCCGCGCCCCCTGCCCATCCCCATCCGAGATAGGGACGA 400

410 420 430 440
GGAGCAGCTGCAGGGAAAGCAGCGAGCGCCGGGAGAGGG 440
GCGGGCAGAAGCGCTGACAAATCAGCGGTGGGGGCGGAGA 480
GCCGAGGAGAAGGAGAAGGAGGAGGACTAGGAGGAGGAGG 520
ACGGCGACGACCAGAAGGGGCCCAAGAGAGGGGGCGAGCG 560
ACCGAGCGCCGCGACGCGAAGTGAGGTGCGTGCGGGCTCA 600

610 620 630 640
GCGCAGACCCCGGCCCGGCCCTCCTGAGAGCGTCCTGGG 640
CGCTCCCTCACGCCTTGCCCTTCAAGCCTTCTGCCTTTCCA 680
CCCTCGTGAGCGGAGAACTGGGAGTGGCCATTCGACGACA 720
GGTTAGCGGGTTTGCCCTCCCACTCCCCAGCCTCGCGTCG 760
CCGGCTCACAGCGGCCTCCTCTGGGGACAGTCCCCCCCCGG 800

810 820 830 840
GTGCCCCCTCCGCCCTTCTGTGCGCTCCTTTTCCTTCTTC 840
TTTCCTATTAAATATTATTTGGGAATTGTTTAAATTTTTT 880
TTTTAAAAAAGAGAGAGGGCGNGGAGGAGTCGGAGTTGTG 920
GAGAAGCAGAGGGACTCAGGTAAGTACCTGTGGATCTAAA 960
CGGGNGTCTTTGGAAATCCTGGAGAACGCCGGATGGAGAC 1000

1010 1020 1030 1040
GAATGGTCGTGGGNACCGGGAGGGGGTGGTGCTGCCATGA 1040
GGACCGCTGGGCCAGGTCTCTGGGAGGTGAGTACTTGTCC 1080
TTTGGGGAGCCTAAGGAAAGAGACTTGACCIGGCTTTTCGT 1120
CCTGCTTCTGATATTCCCTTCTCCACAAGGGCTGAGAGNT 1160
TAGGCTGCTTCTCCGGGATCC 1181



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Figure 11 cont.

alpha-SYN exon 3

```
      10      20      30      40
      |      |      |      |
CTTAAAAGAGTCTCACACTTTGGAGGGTTTCTCATGATTT 40
TTCAGTGTTTTTTGTTTTATTTTTCCCGAAAGTTCTCATT 80
CAAAGTGATTTTTATGTTTTCCAGTGTGGTGTAAGAAAT 120
TCATTAGCCATGGATGTATTCATGAAAGGACTTTCAAAGG 160
CCAAGGAGGGAGTTGTGGCTGCTGCTGAGAAAACCAAACA 200
      210      220      230      240
      |      |      |      |
GGGTGTGGCAGAAGCAGCAGGAAAGACAAAAGAGGGTGTT 240
CTCTATGTAGGTAGGTAAACCCCAAATGTCAGTTTGGTGC 280
TTGTTTCATGAGTGATGGGTTAGGATAACAATACTCTAAAT 320
GCTGGTAGTTCTCTCTCTTGATTCATTTTTGCATCATTGC 360
TTGTCAAAAAGGTGGACTGAGTCAGAGGTATGTGTAGGTA 400
      410      420      430      440
      |      |      |      |
GGTGAATGTGAACGTGTGTATNTGAGCTAATAGTAAAAAT 440
GCGACTGTTTGCTTTTCAGATTTTAAATTTTGCCTAATAT 480
NTATGACTTNTTAAAATGAATGTTTCTGTACTACATAATT 520
CTATNTCAGAGACAGT 536
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Figure 11 cont.

alpha-SYN exon 4

10 20 30 40
CTGCAGGTCAACGGATCTGTCTCTAGTGCTGTACTTTTAA 40
AGCTTCTACAGTTCTGAATTCAAAATTATCTTCTCACTGG 80
GCCCCGGTGTATCTCATTCTTTTTTCTCCTCTGTAAGTT 120
GACATGTGATGTGGGAACAAAGGGGATAAAGTCATTATTT 160
TGTGCTAAAATCGTAATTGGAGAGGACCTCCTGTTAGCTG 200

210 220 230 240
GGCTTTCTTCTATNTATTGTGGTGGTTAGGAGTTCCTTCT 240
TCTAGTTTTAGGATATATATATATATTTTTTTCTTTCCCT 280
GAAGATATAATAATATATATACTTCTGAAGATTGAGATTT 320
TTAAATTAGTTGTATTGAAACTAGCTAATCAGCAATTTA 360
AGGCTAGCTTGAGACTTATGTCTTGAATTTGTTTTTGTAG 400

410 420 430 440
GCTCCAAAACCAAGGAGGGAGTGGTGCATGGTGTGGCAAC 440
AGGTAAGCTCCATTGTGCTTATATCAAAGATGATATNTAA 480
AGTATCTAGTGATTAGTGTGGCCAGTATCAAGATTCCTA 520
TGAAATTGTAAACAATCACTGAGCATCTAAGAACATATC 560
AGTCTTATTGAAACTGAATTCTTTATAAAGTATTTTTTAA 600

610 620 630 640
TAGGTAAATATTGATTATAAATAAAAAATATACTTGCCAA 640
GAATAATGAG 650



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Figure 11 cont.

alpha-SYN exon 5

10 20 30 40
ATATCTTAGCCAAGATTCAATGTTTGGTTGAACCACACTC 40
ACTTGACATCTTGGTGGCTTTTGTCTTCTTGACCACTCA 80
GTTATCTATGGCATGTGTAGATACAGGTGTATGGAANCGA 120
TGGCTAGTGGAAGTGGAATGATTTTAAGTCACTGTTATTC 160
TACCACCCTTTAATCTGTTGTTGCTCTTTATTTGTACCAG 200

210 220 230 240
TGGCTGAGAAGACCAAAGAGCAAGTGACAAATGTTGGAGG 240
AGCAGTGGTGACGGGTGTGACAGCAGTAGCCCAGAAGACA 280
GTGGAGGGAGCAGGGAGCATTGCAGCAGCCACTGGCTTTG 320
TCAAAAAGGACCAGTTGGGCAAGGTATGGCTGTGTACGTT 360
TTGTGTTACATTTATAAGCTGGTGAGATTACGGTTCATTT 400

410 420 430 440
TCATGTGAAGCCTGGAGGCAGGAGCAAGATACTTACTGTG 440
GGGAACGGCTACCTGACCCTCCCCTTGTGAAAAAGTGCTA 480
CCTTTATATTGGTCTTGCTTGTTT 504



Figure 11 cont.

alpha-SYN exon 6

10 20 30 40

AAAAGTTTACATACTTTGAGGTTGATAACCCATGTTGCCG 40
CAATGTTTCCCCGGAGGCATTGTGGAGTTTAGAATGCCAG 80
TAGTAATATTAAGGTGTGCCATTTTCAAGATCCGTGGCCA 120
ACATCCCTATATGTAAGATTTTCCAAAACATGGTTCTGA 160
TTTTTAAAAGTGAAAAATGCTACTTCATCATGTTCTTTT 200

210 220 230 240

GTGCTTCTTACTTTAAATATTAGAATGAAGAAGGAGCCCC 240
ACAGGAAGGAATTCTGGAAGATATGCCTGTGGATCCTGAC 280
AATGAGGCTTATGAAATGCCTTCTGAGGTAGGAGTCCAAG 320
CTGAATCTTTCTAACAAGACAGTACCAAAAACCTGTCATT 360
GTCACATTTCTCTTTTCATTAGTGCTTAGTGAGAATCATTT 400

410 420 430 440

GCTCTCTACATGCTCATTACGTGGACAACCTTGCAAGTTAA 440
GAATAGTTTTTACATTTTTTAAAGGGTCCTTAAAAAAAAAAG 480
AGGAGGAGGAAGATGAAGAAGAGGAAGAAAGGATGTAAAA 520
GAAATCATATGTAGTCCACATAGCTTAATATACNTACTAC 560
TTGACCCTTTACAGGAAAAGCTTTACTAACCCTGCATTA 600

610 620 630 640

GAGAATATATTTTTTTTGCAAAAACATTGATTGTAAATTTT 640
AGTGTAAGTGGGGAGCCATTTCTATCTCATTGGCTGTC 680
CAGTGCTGATGCGTAATTGAAACTTATACTAACAGTGTGT 720
GCTGTCT 727



alpha-SYN exon 7

10 20 30 40
TTTTGATTTTCTAATATTAGGAAGGGTATCAAGACTACG 40
AACCTGAAGCCTAAGAAATATCTTTGCTCCCAGTTTCTTG 80
AGATCTGCTGACAGATGTTCCATCCTGTACAAGTGCTCAG 120
TTCCAATGTGCCCAGTCATGACATTTCTCAAAGTTTTTAC 160
AGTGTATCTCGAAGTCTTCCATCAGCAGTGATTGAAGCAT 200
210 220 230 240
CTGTACCTGCCCCCACTCAGCATTTCGGTGCTTCCCTTTC 240
ACTGAAGTGAATACATGGTAGCAGGGTCTTTGTGTGCTGT 280
GGATTTTGTGGCTTCAATCTACGATGTTAAAACAAATTAA 320
AAACACCTAAGTGACTACCACCTTATTTCTAAATCCTCACT 360
ATTTTTTTGTGCTGTTGTTTCAAGAAGTTGTTAGTGATTG 400
410 420 430 440
CTATCATATATTATNAGATTTTTAGGTGTCTTTTAATGAT 440
ACTGTCTAAGAATAATGACGTATTGTGAAATTTGTTAATA 480
TATATNATACTTAAAAATATGTGAGCATGAAACTATGCAC 520
CTATAATACTAAATATGAAATTTTACCATTTTTCGATGTG 560
TTTTATTCACTTGTGTTTGTATATNAATGGTGAGAATTAA 600
610 620 630 640
AATAAACGTTATCTCATTGCAAAAATATTTTATTTTAT 640
CCCATCTCACTTTAATAATAAAAAATCATGCTTATAAGCAA 680
CATGAATTAAGAACTGACACAAAGGACAAAAATATAAAGT 720
TATTAATAGCCATTTGAAGAAGGAGGAATTTTAGAAGAGG 760
TAGAGAAAATGGAACATTAACCCTACACTCGGAATTCCCT 800
810 820 830 840
GAAGCAACACTGCCAGAAGTGTGTTTTGGTATGCACTGGT 840
TCCTTAAGTGGCTGTGATTAATTATTGAAAGTGGGGTGTT 880
GAAGACCCCACTACTATTGTAGAGTGGTCTATTTCTCCC 920
TTCAATCCTGTCAATGTTTGCTTTACGTATTTTGGGGAAC 960
TGTGTTTGATGTGTATGTGTTTATAATTGTTATACATTT 1000
1010 1020 1030 1040
TTAATTGAGCCTTTTATTAACATATATTGTTATTTTGTG 1040
TCGAAATAATTTTTTAGTTAAAATCTATTTGTCTGATAT 1080
TGGTGTGAATGCTGTACCTTTCTGACAATAAATAATATNC 1120
GACCATGAATAAAAAAAAAAAAAAGTGGGTTCCTGGGAA 1160
CTAAGCAGTGTAGAAGATGATTTTGACTACACCCTCCTTA 1200



Figure 11 cont.

alpha-SYN exon 7

1210	1220	1230	1240
GAGAGCCATAAGACACATTAGCACATATTAGCACATTCAA 1240			
GGCTCTGAGAGAATGTGGTTAACTTTGTTTAACTCAGCAT 1280			
TCCTCACTTTTTTTTTTTAATCATCAGAAATTCTCTCTCT 1320			
CTCTCTCTTTTTCTCTCGCTCTCTTTTTTTTTTTTTTTTT 1360			
TTTTACAGGAAATGCCTTTAAACATCGTTGGGAACCTACCA 1400			
1410	1420	1430	1440
GAGTCACCTTAAAGGGGAGNATCAATTCTCTAGGACTGGAT 1440			
AAAAATTTTCATGGGCCTCCTTTAAAATGTTGCCCAAATAT 1480			
ATGGAATTCTAGGGGTTTTTCCNTAGGGGGAAGGGTTTTT 1520			
TCTCTTTTCNGGGGAGGATCCTTTTAACNCCCCNGGGGGG 1560			
NGCCCGGAAAATAAACTTGGNGGGGGGGNAAAACCTT 1596			



INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/13071

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/47 C12N15/11 C07K16/18 A61K48/00
 C1201/68 G01N33/68 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K C12Q G01N A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document (with indication, where appropriate, of the relevant passages)	Relevant to claim No.
X	SCHAPIRA A. H.: "Pathogenesis of Parkinson's disease." BAILLERES CLINICAL NEUROLOGY, vol. 6, no. 1, April 1997, pages 15-36. XP002083889	1-23, 57-61, 74
Y	see page 17, paragraph 2 see abstract ---	24-56, 62-73
Y	US 5 494 794 A (WALLACE DOUGLAS C) 27 February 1996 see the whole document ---	24-56, 62-73
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☒ Further documents are listed in the continuation of box C☒ Patent family members are listed in annex

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European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel: (+31-70) 340-2040, Tx: 31 651 epo nl
 Fax: (+31-70) 340-3016

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/13071

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication where appropriate of the relevant passages	Relevant to claim No
A	JAKES R. ET AL.: "Identification of two distinct synucleins from human brain." FEBS LETTERS, vol. 345, 1994, pages 27-32, XP002078475 cited in the application & UEDA K. ET AL.: "Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease." PROC. NATL. ACAD. SCI. USA, vol. 90, 1993, pages 11282-11286, see figure 2 ---	1-74
A	CHEN X. ET AL.: "The human NACP/alpha-synuclein gene: chromosome assignment to 4q21.3-q22 and TaqI RFLP analysis." GENOMICS, vol. 26, no. 2, 1995, pages 425-427, XP002083890 cited in the application ---	1-74
A	POLYMEROPOULOS M. H. ET AL.: "Mapping of a gene for Parkinson's disease to chromosome 4q21-q23." SCIENCE, vol. 274, 1996, pages 1197-1199, XP002083891 cited in the application see the whole document ---	1-74
A	MAROTEAUX L. AND SCHELLER R. H.: "The rat brain synucleins: family of proteins transiently associated with neuronal membrane." MOLECULAR BRAIN RESEARCH, vol. 11, 1991, pages 335-343, XP002083892 cited in the application see figure 1 ---	1-74
P,X	NUSSBAUM R. L. AND POLYMEROPOULOS M. H.: "Genetics of Parkinson's disease." HUMAN MOLECULAR GENETICS, vol. 6, no. 10, 1997, pages 1687-1691, XP002083893 see the whole document ---	1-74
P,X	GOEDERT M.: "The awakening of alpha-synuclein." NATURE, vol. 388, 17 July 1997, pages 232-233, XP002083894 see the whole document ---	1-74

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INTERNATIONAL SEARCH REPORT

International Application No

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C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No
P.X	<p>POLYMEROPOULOS M. H. ET AL.: "Mutation in the alpha-synuclein gene identified in families with Parkinson's disease."</p> <p>SCIENCE, vol. 276, 27 June 1997, pages 2045-2047, XP002083895 see the whole document -----</p>	1-74



Information on patent family members

PC//US 98/13071

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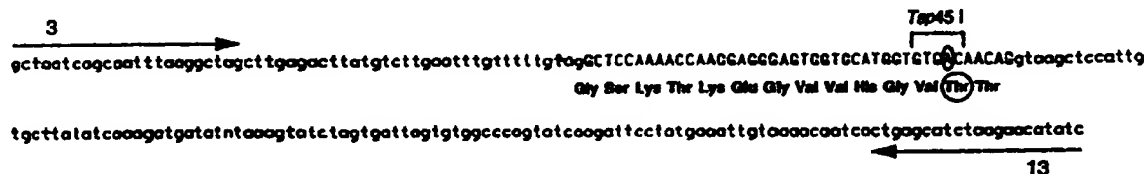




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(21) International Application Number: PCT/US98/13071 (22) International Filing Date: 25 June 1998 (25.06.98) (30) Priority Data: 60/050,684 25 June 1997 (25.06.97) US		201, 14020 Old Harbor Lane, Marina Del Ray, CA 90292 (US). (74) Agents: SCHNELLER, John, W. et al.; Spencer & Frank, Suite 300 East, 1100 New York Avenue, N.W., Washington, DC 20005-3955 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Office of Technology Transfer, National Institute of Health, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): POLYMEROPOULOS, Mihael, H. [US/US]; 8301 Raymond Lane, Potomac, MD 20854 (US). LAVEDAN, Christian [FR/US]; 14421 Frances Green Way, North Potomac, MD 20878 (US). LEROY, Elisabeth [FR/US]; 4316 Garrison Street, N.W., Washington, DC 20016 (US). NUSSBAUM, Robert, L. [US/US]; 3815 Leland Street, Chevy Chase, MD 20815 (US). JOHNSON, William, G. [US/US]; 91 Stewart Road, Short Hills, NJ 07078 (US). DUVOISIN, Roger, C. [US/US]; Apartment		Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	

(54) Title: CLONING OF A GENE MUTATION FOR PARKINSON'S DISEASE



(57) Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder with a lifetime incidence of approximately 2 percent. It was recently reported that a PD susceptibility gene is located on the long arm of human chromosome four. The present invention reports the subsequent identification of a mutation in the alpha synuclein gene, which codes for a presynaptic protein thought to be involved in neuronal plasticity. The finding of a specific molecular alteration which is causative for PD will permit the detailed understanding of the pathophysiology of the disorder, which will lead to potential therapeutic interventions, as well as a means for diagnosing individuals having an increased risk of developing the disease.

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CLONING OF A GENE MUTATION FOR PARKINSON'S DISEASE

5 BACKGROUND OF THE INVENTION

1. Field of the Invention

Parkinson's disease (PD) is a common neurodegenerative disorder with a lifetime incidence of approximately 2
10 percent. A pattern of familial aggregation has been documented for the disorder, and it was recently reported that a PD susceptibility gene in a large Italian kindred is located on the long arm of human chromosome 4. We have identified a mutation in the alpha synuclein gene, which
15 codes for a presynaptic protein thought to be involved in neuronal plasticity, in the Italian kindred and in three unrelated families of Greek origin with autosomal dominant inheritance for the PD phenotype. This finding of a specific molecular alteration which is causative for PD will permit
20 the detailed understanding of the pathophysiology of the disorder. In addition, methods of screening nucleic acids for the presence of mutations in the synuclein gene to test for predisposition to Parkinson's Disease are now possible.

25 2. Technology Background

Parkinson's disease (PD) was first described by James

Parkinson in 1817 (1). The clinical manifestations of this neurodegenerative disorder include resting tremor, muscular rigidity, bradykinesia and postural instability. A relatively specific pathological feature accompanying the neuronal degeneration is the intracytoplasmic inclusion body, known as the Lewy body, which is found in many regions including the substantia nigra, locus ceruleus, nucleus basalis, hypothalamus, cerebral cortex, cranial nerve motor nuclei, and the central and peripheral divisions of the autonomic nervous system (1).

In many cases a heritable factor predisposes to the development of the clinical syndrome (2). We have recently shown that genetic markers on human chromosome 4q21-q23 segregate with the PD phenotype in a large family of Italian descent (3). The clinical picture of the PD phenotype in the Italian kindred has been well documented to be typical for PD, including Lewy bodies, with the exception of a relatively earlier age of onset of illness at 46 ± 13 years. In this family the penetrance of the gene has been estimated to be 85%, suggesting that a single gene defect is sufficient to determine the PD phenotype.

We now report the identification of a mutation in the alpha synuclein gene that is associated with Parkinson's disease. The mutation, an Ala53Thr substitution, was found to be linked to the PD phenotype in four independent PD

families and absent from 314 control chromosomes, providing strong genetic evidence that this mutation in the human alpha synuclein gene is causative for the PD phenotype in these families.

5 The Ala53Thr substitution is localized in a region of the protein whose secondary structure predicts an alpha helical formation, bounded by beta sheets. Substitution of the alanine with threonine is predicted to disrupt the alpha helix and extend the beta sheet structure. Beta pleated
10 sheets are thought to be involved in the self aggregation of proteins which could lead to the formation of amyloid like structures (6).

 This was already tested in the case of NAC35, the 35 amino acid peptide derived from alpha-synuclein that was
15 first isolated from plaques found in patients with Alzheimer's disease (4). NAC35 was shown to self aggregate and form amyloid fibril which shared the 'amyloid' characteristics of insolubility in aqueous solutions and green birefringence under polarized light, subsequent to
20 Congo red staining (6). NAC35 is located in the middle of the alpha synuclein molecule and extends from amino acid 61 to amino acid 95. Residue 53, which is found to be mutated in PD, is outside the NAC35 peptide found in amyloid
 plaques. However, the true size of the NAC peptide involved
25 in the plaques is not known since the protease used to

isolate the peptide from AD tissue cuts at lysine 60 of the alpha synuclein protein. It is therefore possible that amino acid 53 may be part of the NAC peptide found in plaques. In crosslinking experiments with beta amyloid (Abeta), it was demonstrated (6) that residues 1-56 and 57-97 specifically bind amyloid and that a synthetic peptide consisting of residues 32-57 performed similarly.

Three members of the synuclein family have been characterized in the rat, with SYN1 exhibiting 95% homology with the human alpha-synuclein protein (7). SYN 1 of the rat is expressed in many regions of the brain, with high levels found in the olfactory bulb and tract, the hippocampus, dentate gyrus, habenula, amygdala and piriform cortex, and with intermediate levels in the granular layer of the cerebellum, substantia nigra, caudate-putamen, and dorsal raphe (7). This pattern of expression coincides with the distribution of the Lewy bodies found in brains of patients with Parkinson's disease. It is also interesting to note that decrease in olfactory sense often accompanies the syndromic features of Parkinson's disease, and in many cases it is proposed that hyposmia is a prodromic sign of the illness (8).

In the zebra-finch the homologue to alpha synuclein, synelfin, is thought to be involved in the process of song learning, suggesting a role for synuclein perhaps in memory

and learning (9). In contrast to humans, rats have a threonine at residue 53 of their homologues to the human alpha synuclein gene (Figure 4). Similarly, the zebra-finch synelfin carries a threonine at amino acid 53, whereas both Bos taurus and Torpedo californica do not (10). There are no reports that suggest the presence of Lewy bodies in the brains of the rat or the zebra finch or a phenotype resembling that of PD. Lack of any phenotype could be explained by a combination of factors, including the following: the relative short life span of rodents may prohibit the observation of a late onset disorder, interaction with other cellular components not present in the rat may be required for the phenotype, absence of a critical environmental trigger in the rodents, or finally a heterozygous status Ala/Thr may be necessary for the production of a phenotype.

Studies of early onset AD have previously documented that missense mutations can cause an adult onset neurodegenerative disorder. Of the 31 mutations described so far in the loci for presenilin 1 and 2, thirty were missense and one was a splice variant (11). Missense mutations in the prion protein have also been implicated in the amyloid production seen in Gerstmann-Straussler-Scheinker and Creutzfeld-Jakob diseases, both forms of spongiform encephalopathy (12). Studies in these neurodegenerative

disorders have pointed to the importance of the physical
chemical properties of mutant cellular proteins in initiating
and propagating neuronal lesions leading to disease. Similar
studies in the synuclein protein family may provide valuable
5 insights into the etiology and pathogenesis of PD.

Similarly with the mutations in the presenilin genes in
patients with early onset Alzheimer's disease, the mutation
identified in the alpha synuclein gene is unlikely to account
for the majority of sporadic and familial cases of PD.

10 However, this mutation may account for a significant
proportion of those families with a highly penetrant, early
onset autosomal dominant PD phenotype.

All publications and patent applications herein are
15 incorporated by reference to the same extent as if each
individual publication or patent application was specifically
and individually indicated to be incorporated by reference.

3. Summary of the Invention

20 As described herein, we have discovered that particular
mutations in the alpha synuclein gene are associated with
predisposition to Parkinson's disease. Accordingly, the
present invention includes an isolated nucleic acid
comprising a mutated synuclein gene. In particular, the
25 isolated nucleic acid of the present invention contains at

least one mutation in the alpha synuclein gene at base pair position 209 of Genbank # L08850, which, in particular, is a change from guanine to adenine. However, since other mutations in the alpha synuclein gene may also lead to Parkinson's Disease (PD), other mutations are also included. In addition, it is conceivable that mutations in the related beta (46) (SEQ ID NO 11) and gamma (SEQ ID NOS 12 and 13) synuclein genes may also lead to PD. Thus, mutated homologues of the alpha synuclein gene are also included in the present invention. Vectors comprising the isolated nucleic acid and host cells comprising such vectors are included as well.

Knowledge of particular genes that are associated with PD allows for the search for other specific PD mutations. Accordingly, the present invention also includes a method of using a synuclein gene sequence to identify specific PD mutations. Such mutations may occur in an unrelated population or in a family that demonstrates passage of PD within the family tree.

Since knowledge of mutations associated with Parkinson's disease allows the development of genetic screens that test for an individual's chances of being predisposed to the disease, and such tests may be performed by hybridization analysis using oligonucleotides complementary to the sequence of interest or by PCR amplification using oligonucleotides that are complementary to sequences flanking the mutation, the present invention also includes oligonucleotides

complementary to a portion of the synuclein gene, wherein said portion comprises or flanks a mutation associated with predisposition to Parkinson's Disease. In particular, the oligonucleotides of the present invention will have a sequence that is complementary to a sequence from the alpha synuclein gene that includes or flanks base pair position 209. And in particular, this mutation is a change from guanine to adenine at this position.

Vectors comprising an isolated nucleic acid encoding a mutated synuclein gene will allow the production and isolation of the mutant protein in an appropriate host cell using techniques well known in the art. Alternatively, peptides may be chemically synthesized using techniques also well known in the art. Isolation of such a protein or peptides thereof will allow the study of the molecular mechanisms which lead to development of Parkinson's disease. Accordingly, the present invention also includes an isolated synuclein protein or peptide containing at least one mutation. In particular, this mutation is at a position corresponding to the fifty-third amino acid in the native alpha synuclein protein, and in particular, this mutation is an alanine to threonine substitution.

Peptides corresponding to portions or the entirety of a synuclein gene may be useful as drugs for inhibiting the self-aggregation of mutant proteins that is thought to lead to Parkinson's disease. Accordingly, the present invention includes a method of testing peptides and other compounds for

the ability to interfere with this self-aggregation. Self-aggregation can be tested using a number of established methods, including Congo red staining, electron microscopy pictures of amyloid fibrils, and circular dichroism (CD) spectrophotometry. Using a peptide derived from the alpha synuclein protein that includes the mutant THR amino acid at position 53 alone or in combination with a normal peptide may allow testing for drugs that can inhibit the aggregation or dissolve an aggregate. This procedure can be used to rapidly identify agents that could be used in animal studies, clinical trials, or as diagnostic tools.

Possession of isolated synuclein proteins or peptides will also allow the isolation of specific antibodies using techniques well known in the art. Such antibodies may distinguish a mutant synuclein protein from its wildtype counterpart, and therefor could also be used in diagnostic screens. Alternatively, such antibodies may also be used to inhibit the self-aggregation of proteins during the progression of Parkinson's disease. Accordingly, the present invention also includes antibodies specific for a mutated synuclein protein or peptide. It should be understood that useful derivatives of such antibodies, such as Fv fragments and Fab fragments, are also included.

The above aspects of the present invention will allow methods of detecting subjects at increased risk for Parkinson's Disease. Such a method comprises obtaining a sample comprising nucleic acids from the subjects, and

detecting in the nucleic acids the presence of a mutation which is associated with Parkinson's disease. In particular, the mutation detected by the method of the present invention is located on human chromosome four, preferably in the alpha synuclein gene. In particular, the mutation causes an amino acid substitution at position 53 of the alpha synuclein gene, which is, in particular, an alanine to threonine substitution.

The detecting step of the method of the present invention may be accomplished several different ways as will be described in further depth below. All such methods are well known to those of ordinary skill in the art.

For instance, said detecting step may comprise combining a nucleotide probe which selectively hybridizes to a nucleic acid containing a mutation associated with a predisposition to Parkinson's disease, and detecting the presence of hybridization. Such a probe may be an oligonucleotide that is complementary to a portion of the synuclein gene, wherein said portion comprises the mutation. In particular, such an oligonucleotide is complementary to a mutated alpha synuclein gene having at least one mutation at base pair position 209. In particular, this mutation is a change from guanine to adenine.

The detecting step of the method of the present invention may also comprise amplifying a nucleic acid product comprising said mutation, and detecting the presence of said mutation in the amplified product using any nucleic acid

sequencing procedure known in the art. Alternatively, the detecting step may comprise selectively amplifying a nucleic acid product comprising said mutation, and detecting the presence of amplification using any appropriate method known in the art. Such methods include gel electrophoresis of amplified nucleic acids, and detection of radiolabeled amplified nucleic acids using autoradiographic film or any other detection method known in the art.

The amplifying step of the present invention may be performed using the polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR), or any other type of PCR reaction known in the art. Accordingly, such a step will comprise at least one annealing step whereby at least one oligonucleotide is annealed to said sample of nucleic acids. In particular, said amplifying step uses two oligonucleotides. And in particular, the two oligonucleotides have the sequences given in SEQ ID NOS 2 and 3.

Alternatively, the detecting step of the method of the present invention comprises detecting the presence or absence of a restriction endonuclease site as detected by enzymatic digest of a nucleic acid sample. Such a detecting means will be possible when a mutation associated with a predisposition to Parkinson's disease results in a sequence having a new restriction endonuclease cleavage site, or loss of a native restriction endonuclease site. In particular, the mutation associated with Parkinson's disease results in the formation of a non-native *Tsp45I* restriction endonuclease site.

Alternatively, the detecting step of the present invention may be performed using a gene-specific primer and subsequent chain termination at the position of the mutation using DNA polymerase and labeled nucleotides or
5 dideoxynucleotides. The presence of nucleic acids in which a dideoxynucleotide corresponding to the mutation of interest is incorporated at the appropriate position may be detected by any means known in the art, including detection of radiolabeled dideoxynucleotides using, for example,
10 autoradiographic film, or detection of fluorescently-labeled dideoxynucleotides.

Since the methods and compounds of the present invention will be useful in diagnostic screening procedures aimed at identifying individuals having a predisposition for
15 Parkinson's disease, the present invention also includes diagnostic kits which include the compounds of the present invention in a form that allows such compounds to be used quickly and easily for the designated purpose.

Finally, the inventors also contemplate that the
20 isolated nucleic acid, oligonucleotides and antibodies of the present invention may eventually be used in methods directed at the correction or suppression of Parkinson's disease. For example, oligonucleotides or expression vectors designed from the synuclein nucleic acid sequences of the present invention
25 may one day be used in antisense therapy directed at inhibiting expression of the mutated synuclein protein in

patients with Parkinson's disease, or in individuals having a predisposition for Parkinson's disease. Similarly, antibodies specific for the mutated synuclein protein may be useful in therapies directed at inhibiting the self-aggregation of mutated proteins or peptides in patients having Parkinson's disease. Knowledge of gene(s) associated with the development of Parkinson's disease may also allow the design of transgenic animals which express the mutant gene(s). Such animals may serve as a useful disease model, allowing one to test the effects of candidate therapies and therapeutic compositions in the treatment or inhibition of Parkinson's disease.

A detailed description of the present invention is now provided, and should not be considered as limiting on the present invention as described above.

4. Brief Description of the Drawings

Figure 1.

DNA sequence of the PCR product used for mutation detection (SEQ ID NO 1). Oligonucleotide primers are shown by arrows and the numerals 3 and 13 (SEQ ID NO 2 and 3). Intron sequence is shown in lower case and exon sequence in upper case. Amino acid translation of the exon is shown below the DNA sequence. The circled base represents the G209A change in the mutant allele. The resulting amino acid

Ala53Thr change is represented by the circled amino acid. The newly created *Tsp45* I site is indicated above the DNA sequence.

5 Figure 2.

 Mutation analysis of the G209A change is shown in a subpedigree of the Italian kindred. Filled symbols represent affected individuals. Numerical identifiers, denote the individuals immediately above. *Tsp45* I digestion of PCR products is shown at the bottom of the figure, and fragment sizes are indicated on the right in base pairs.

10

 Figure 3.

 Mutation analysis of the G209A change in RT PCR products (7). Lane 1: 100 bp ladder, lanes 2 and 3 normal control, lanes 4 and 5 PD patient, lane 6 negative control without RT enzyme. Sizes are indicated on the right in base pairs. Lanes 2 and 4 show uncut DNA and lanes 3 and 5 show DNA cut with *Tsp45* I.

15

20

 Figure 4.

 Sequence alignments of alpha synuclein homologues in different species. Accession numbers for the sequences used were as follows: Homo sapiens Swiss-Prot P37840 (SEQ ID NO 4), Rattus norvegicus Swiss-Prot P37377 (SEQ ID NO 5), Bos

25

taurus Swiss-Prot P33567 (SEQ ID NO 6), *Serinus canaria*
genbank L33860 (SEQ ID NO 7), *Torpedo californica* Swiss-Prot
P37379 (SEQ ID NO 8). Numbering on top of the alignments is
according to the human sequence. Amino acid 53, which is the
5 site of the Ala53Thr change, is circled.

Figure 5.

The pedigree of a large family with PD (3). The
clinical and pathological features of some members of this
10 kindred were previously reported.

Figure 6.

Multipoint LOD score analysis between chromosome 4q
markers and the PD locus.

15

Figure 7.

A table of human synuclein clones identified from
various databases. Columns labeled 5' and 3' show the
sequence acquisition numbers. Clones were identified by
20 homology to protein or nucleic acid sequence. Human gamma
clones were identified by homology to known mouse and rat
gamma synuclein sequences.

Figure 8.

25 Sequence of BAC clone 139A20 for human beta synuclein.

BAC clone was isolated using primers to known database sequences described in Figure 7. The sequence shown includes all coding exon sequences and some non-coding intronic sequences. (SEQ ID NO:11)

5

Figure 9.

Sequence from the 5' end of BAC clone 174P13 for human gamma synuclein. The BAC clone was isolated with primers from the database sequences described in Figure 7. (SEQ ID

10 NO:12)

Figure 10.

Sequence from the 3' end of BAC clone 174P13 for human gamma synuclein. BAC clone was isolated as described in Figure 9. The entire human gamma synuclein gene has now been sequenced and has been deposited in GenBank: accession number AF044311. (SEQ ID NO: 13)

15

Figure 11.

Sequence of exons 1-7 of the human alpha synuclein gene, plus some flanking intronic sequence for each exon. (SEQ ID NOs 14-19)

20

5. Detailed Description of the Invention

Definitions

25

Unless defined otherwise, all technical and scientific

terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. For purposes of the present invention, the following terms are defined below.

This invention provides a method of diagnosing or predicting a predisposition to Parkinson's disease. The method comprises detecting in a sample from a subject the presence of a mutation, for example, in nucleotide position 209 of the human alpha synuclein gene. The presence of the mutation indicates the presence of or a predisposition to Parkinson's disease.

As used herein, the term "gene" primarily relates to a coding sequence, but can also include some or all of the surrounding or flanking regulatory regions or introns. The term "gene" specifically includes artificial or recombinant genes created from cDNA or genomic DNA, including recombinant genes based upon splice variants.

As used herein, the term "synuclein" gene or protein may refer to the alpha synuclein gene or any homologue thereof. A "homologue" is understood to mean any related gene or protein that is at least 25% homologous to the alpha synuclein gene or protein or performs a related function. Preferably, a synuclein gene or protein refers to alpha, beta or gamma synuclein, but most preferably refers to alpha

synuclein.

As used herein, an "isolated nucleic acid" is a ribonucleic acid, deoxyribonucleic acid, or nucleic acid analog comprising a polynucleotide sequence that has been isolated or separated from sequences that are immediately contiguous, i.e. on the 5' and 3' ends, in the naturally occurring genome of the organism from which it is derived. The term therefor includes, for example, a recombinant nucleic acid which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule independent from any other sequences.

An isolated nucleic acid of the present invention may be "operatively linked" to an expression control sequence or regulatory region. As used herein, "operatively linked" means that the components are joined in such a way that the expression, transcription or translation of the sequence is under the influence or control of the regulatory region.

As used herein, a "predisposition" to Parkinson's disease means an increased probability of developing Parkinson's disease during the subject's lifetime as compared to the average individual.

Pertaining to this probability, a LOD score is a measure of genetic linkage used herein, defined as the \log_{10} ratio of the probability that the data would have arisen if the loci are linked to the probability that the data could have arisen from unlinked loci. The conventional threshold for declaring

linkage is a LOD score of 3.0, that is, a 1000:1 ratio (which must be compared with the 50:1 probability that any random pair of loci will be unlinked).

As used herein, reference to "base pair position" or "amino acid position" when referring to an isolated nucleic acid, probe, protein or peptide always indicates the relative position in the native gene or protein.

A "probe" refers to a nucleic acid which has sufficient nucleotides surrounding the codons at the mutation positions to distinguish the nucleic acid from nucleic acids encoding non-related genes. The specific length of the nucleic acid is a matter of routine choice based on the desired function of the sequence. For example, if one is making probes to detect the mutation in base pair position 209, the length of the nucleic acid is preferably small, but must be long enough to prevent hybridization to undesired background sequences. However, if the desired hybridization is to a nucleic acid which has been amplified, background hybridization is less of a concern and a smaller probe can be used. In general, such a probe will be between 10 and 100 nucleotides, especially between 10 and 40 and preferably between 15 and 25 nucleotides in length. It is apparent to one of skill in the art that nucleotide substitutions, deletions, and additions may be incorporated into the polynucleotides of the invention. However, such nucleotide substitutions, deletions, and additions should not substantially disrupt the ability of the polynucleotide to hybridize under conditions that are

sufficiently stringent to result in specific hybridization.

As used herein with respect to genes, "the term "normal" refers to a gene which encodes a normal protein. As used herein with respect to proteins, the term "normal" means a protein which performs its usual or normal physiological role and which is not associated with, or causative of, a pathogenic condition or state. Therefor, the term "normal" is generally synonymous with the phrase "wild type".

For any given gene or corresponding protein, a multiplicity of normal allelic variants may exist, none of which is associated with the development of a pathogenic condition or disease state. Such normal allelic variants include, but are not limited to, variants in which one or more nucleotide substitutions do not result in a change in the encoded amino acid sequence.

As used herein, the term "mutation" generally refers to a mutation in a gene that is associated with a predisposition to Parkinson's disease. "Mutant" can specifically refer to a mutation at nucleotide position 209 of the synuclein gene, and is in particular a G to A transition. However, other mutations in the synuclein gene or other genes which are associated with a predisposition to Parkinson's disease are also encompassed. Furthermore, the term "mutation" is not limited to transition mutations, but can also mean a deletion, insertion or transversion as well.

The term "mutant", as it applies to synuclein genes, is not intended to embrace sequence variants which, due to the

degeneracy of the genetic code, encode proteins identical to the normal sequences disclosed or otherwise enabled herein; nor is it intended to embrace sequence variants which, although they encode different proteins, encode proteins which are functionally equivalent to normal synuclein proteins. The term "mutant" means a protein which does not perform its usual or normal physiological role and which is associated with, or causative of, a pathogenic condition or state.

Since a mutation can be a substitution, deletion or insertion, a mutated synuclein "protein" is understood to refer to the amino acid sequence resulting from any such mutation whether the resulting protein is shorter, longer or modified, i.e. due to an alteration in reading frame or generation of stop codon. In addition, "peptide" is understood to refer to a portion of the mutated protein that is preferably at least five base pairs long, and more preferably at least 10 base pairs long. This portion may be derived from the amino or carboxyl terminus, or it may be an internal portion of the full length protein. As such, a peptide may be chemically synthesized using any method known in the art, or may be made using a recombinant DNA technology and an appropriate purification scheme or isolated from the native protein using enzymatic digestion.

As used herein, the term "substantially pure" means a preparation which is at least 60% by weight the compound of interest. Preferably the preparation is at least 75%, more

preferably 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate method, i.e. column chromatography, gel electrophoresis or HPLC analysis.

5 "Specific or selective hybridization" as used herein means the formation of hybrids between a probe nucleic acid (e.g., a nucleic acid which may include substitutions, deletions, and/or additions) and a specific target nucleic acid (e.g., a nucleic acid having the mutated sequence),
10 wherein the probe preferentially hybridizes to the specific target such that, for example, a band corresponding to the mutated DNA or restriction fragment thereof can be identified on a Southern blot, whereas a corresponding normal or
15 wild-type DNA is not identified or can be discriminated from a variant DNA on the basis of signal intensity. Hybridization probes capable of specific hybridization to detect a single-base mismatch may be designed according to methods known in the art (13-17).

20 "Stringent" as it refers to hybridization conditions is a term of art understood by those of ordinary skill to refer to those conditions of temperature, chaotropic acids, buffer and ionic strength which permit hybridization of a particular nucleic acid sequence to its complementary sequence and not to substantially different sequences. The exact conditions
25 which constitute "stringent" conditions depend on the nature of the nucleic acid sequence, the length of the sequence, and the frequency of occurrence of subsets of that sequence

within other non-identical sequences. By varying hybridization conditions from a level of stringency at which non-specific hybridization occurs to a level at which only specific hybridization occurs, one of ordinary skill in the art can, without undue experimentation, determine conditions which will allow a given sequence to hybridize only with complementary sequences.

Suitable ranges of stringency conditions are described in Sambrook et al. (13). Hybridization conditions, depending on the length and commonality of a sequence, may include temperatures of 20°C-65°C and ionic strengths from 5X to 0.1X SSC. Highly stringent hybridization conditions may include temperatures as low as 40°C-42°C (when denaturants such as formamide are included) or up to 60°C-65°C in ionic strengths as low as 0.1X SSC. These ranges are, however, only illustrative and, depending on the nature of the target sequence, and possible future technological developments, may be more stringent than necessary. Appropriate conditions may be determined for each specific nucleic acid sequence or oligonucleotide probe using standard control and a level of experimentation that is not considered to be undue by those of skill in the art.

As discussed below in greater detail, the mutation can be detected by many methods. For example, the detecting step

can comprise combining a nucleotide probe capable of selectively hybridizing to a nucleic acid containing the mutation with a nucleic acid in the sample and detecting the presence of hybridization. Additionally, the detecting step
5 can comprise amplifying the nucleotides surrounding and including the mutation and detecting the presence of the mutation in the amplified product, or selectively amplifying the nucleotides of the mutation and detecting the presence of amplification. Finally, the detecting step can comprise
10 detecting the presence or absence of a restriction fragment created by an enzyme digest of the sample nucleic acid, or any other detection means known in the art.

Detection Techniques

15 Once the location of a PD-relevant mutation is known, the methods to detect such a mutation are standard in the art. The sequence of various nucleotide probes can be determined from the known sequence of the relevant gene, especially the sequences surrounding the mutation.

20 Detection of point mutations using direct probing involves the use of oligonucleotide probes which may be prepared, for example, synthetically or by nick translation. The probes may be suitably labeled using, for example, a radio label, enzyme label, fluorescent label, biotin-avidin label and the like
25 for subsequent visualization by any appropriate assay, i.e. Southern blot hybridization. In this procedure, the labeled probe is reacted with sample DNA that is bound, for example,

to a nylon filter under conditions such that only fully complementary sequences hybridize. The areas that carry DNA sequences complementary to the labeled DNA probe become labeled themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling may then be visualized, for example, by autoradiography.

Methods of manipulating hybridization conditions to achieve varying degrees of specificity are well known in the art. For example, tetra-alkyl ammonium salts may be used to bind selectively to A-T base pairs, thus displacing the dissociation equilibrium and raising the melting temperature. At 3M Me 4NCl, this is sufficient to shift the melting temperature to that of G-C pairs. This results in a marked sharpening of the melting profile. The stringency of hybridization in such an experiment is usually 5°C below the T_i (the irreversible melting temperature of the hybrid formed between the probe and its target sequence) for the given chain length. For a 20mer oligonucleotide probe, the recommended hybridization temperature is about 58°C. The washing temperatures are unique to the sequence under investigation and need to be optimized for each variant.

There are certainly other ways known in the art for adjusting hybridization conditions in view of desired specificity. For instance, although hybridization may be carried out in accordance with conventional hybridization methods under suitable conditions with respect to e.g. stringency, incubation time, temperature, etc, the choice of

conditions will depend on the desired degree of complementarity between the fragments to be hybridized. A high degree of complementarity requires more stringent conditions such as low salt concentrations, low ionic strength of the buffer and higher temperatures, whereas a low degree of complementarity requires less stringent conditions, e.g. higher salt concentration, higher ionic strength of the buffer or lower temperatures, for the hybridization to take place.

10 The support to which DNA or RNA fragments of the sample to be analyzed are bound in denatured form is preferably a solid support and may have any convenient shape. Thus, it may, for instance, be in the form of a plate, e.g. a thin layer or a microtiter plate, a strip, a solid particle e.g. 15 in the form of a bead such as a latex bead, a filter, a film or paper. The solid support may be composed of a polymer, preferably nylon or nitrocellulose.

Alternative probing techniques, such as ligase chain reaction (LCR), may involve the use of mismatch probes, i.e., 20 probes which are fully complementary with the target except at the point of the mutation. The target sequence is then allowed to hybridize both with oligonucleotides which are fully complementary and have oligonucleotides containing a mismatch, under conditions which will distinguish between the 25 two. By manipulating the reaction conditions according to the above considerations, it is possible to obtain hybridization only where there is full complementarity. If a mismatch is

present there is significantly reduced hybridization.

The polymerase chain reaction (PCR) is a technique that amplifies specific DNA sequences with remarkable efficiency. Repeated cycles of denaturation, primer annealing and

5 extension carried out with Taq polymerase, e.g., a heat stable DNA polymerase, leads to exponential increases in the concentration of desired DNA sequences. Given a knowledge of the nucleotide sequence of the mutations, synthetic

oligonucleotides can be prepared which are complementary to sequences which flank the DNA of interest. Each

10 oligonucleotide is complementary to one of the two strands. The DNA is denatured at high temperatures (e.g., 95°C) and

then reannealed in the presence of a large molar excess of oligonucleotides. The oligonucleotides, oriented with their

15 3' ends pointing towards each other, hybridize to opposite strands of the target sequence and prime enzymatic extension

along the nucleic acid template in the presence of the four deoxyribonucleotide triphosphates. The end product is then

denatured again for another cycle. After this three-step

20 cycle has been repeated several times, amplification of a DNA segment by more than one million-fold can be achieved. The

resulting DNA may then be directly sequenced in order to locate any genetic alteration. Alternatively, it may be

possible to prepare oligonucleotides that will only bind to altered DNA, so that PCR will only result in multiplication

25 of the DNA if the mutation is present. Following PCR, direct visualization or allele-specific oligonucleotide

hybridization (18) may be used to detect the Parkinson's disease point mutation. Alternatively, PCR may be followed by restriction endonuclease digestion with subsequent analysis of the resultant products.

5 As shown in the examples, the substitution of G for A at base pair 209 of the synuclein, results in the gain of a *Tsp45I* site. The gain of this restriction endonuclease recognition site facilitates the detection of the Parkinson's disease mutation using restriction fragment length
10 polymorphism (RFLP) analysis or by detection of the presence or absence of the restriction site in a PCR product that spans base pair position 209.

 For RFLP analysis, DNA is obtained, for example from the blood cells of the subject suspected of having Parkinson's
15 disease and from a normal subject, is digested with a restriction endonuclease, and subsequently separated on the basis of size by agarose gel electrophoresis. The Southern technique can then be used to detect, by hybridization with labeled probes, the products of endonuclease digestion. The
20 patterns obtained from the Southern blot can then be compared. Using such an approach, an additional restriction endonuclease site, such as a *Tsp45I* site, is detected by determining the number of bands detected and comparing this number to the normal subject.

25 The creation of a new restriction site as a result of a nucleotide substitution at a disclosed mutation site can be

readily determined by reference to the genetic code and a list of nucleotide sequences recognized by restriction endonucleases (19).

5 In general, primers for PCR are usually about 20 bp in length, and are most preferably 15-25 bp. Denaturation of strands usually takes place at 94°C. and extension from the primers is usually at 72°C. The annealing temperature varies according to the sequence under investigation. Examples of reaction times are: 20 mins denaturing; 35 cycles of 2 min, 1
10 min, 1 min for annealing, extension and denaturation; and finally a 5 min extension step.

PCR "amplification of specific alleles" (PASA) may also be used to detect the presence of the PD mutation. PASA is a rapid method of detecting single-base mutations or
15 polymorphisms (22-28). PASA (also known as allele specific amplification) involves amplification with two oligonucleotide primers such that one is allele-specific. The desired allele is efficiently amplified, while the other allele(s) is poorly amplified because it mismatches with a
20 base at or near the 3' end of the allele-specific primer. Thus, PASA or the related method of PAMSA may be used to specifically amplify the mutation sequences of the invention. Where such amplification is done on genetic material (or RNA) obtained from an individual, it can serve as a method of
25 detecting the presence of the mutations.

As mentioned above, a method known as ligase chain reaction (LCR) can be used to successfully detect a

single-base substitution (29, 30). LCR probes may be combined or multiplexed for simultaneously screening for multiple different mutations. Thus, LCR can be particularly useful where multiple mutations are predictive of the same disease.

Finally, the Parkinson's disease mutation of the present invention may also be detected using chain termination with labeled dideoxynucleotides. For instance, U.S. Patent No. 5,047,519 to Hobbs et al. discloses fluorescently-labeled nucleotides as chain-terminating substrates for a fluorescence-based DNA sequencing method. With such substrates and knowledge of the gene sequence of interest, it is possible to design an assay using a gene-specific primer to initiate a polymerase reaction immediately flanking the position of the mutation, employing color-coded dideoxynucleotide terminators such that the specific nucleotide at the position of the mutation may be easily determined via a colorimetric assay.

Transgenic Animals and Cell Lines

Having identified subjects having a predisposition to Parkinson's disease associated with a specific mutation, the subjects can participate in the screening of putative agents capable of treating Parkinson's disease. This method comprises administering the test agent to the subject, which may be a human, which has a mutation in a gene associated with Parkinson's disease and monitoring the effect of the

agent on the subject's condition. If the symptoms of Parkinson's disease improve, the agent can be used as a treatment for the disease.

In addition, it is possible to develop transgenic model systems and/or cell lines containing the mutated nucleic acid(s) for use, for example, as model systems for screening for drugs and evaluating drug efficiency. Additionally, such model systems provide a tool for defining the underlying biochemistry of, for instance, the mutated synuclein gene, thereby providing a rationale for drug design.

One approach to creating transgenic animals is to mutate the animal gene of interest by in vivo mutagenesis, transfer the mutant gene into embryonic stem cells by DNA transfection and inject the embryonic stem cells into blastocysts in order to retrieve offspring which carry the disease-causing mutation (31). Alternatively, the technique of microinjection of the mutated gene, into a one-cell embryo followed by incubation in a foster mother can be used. Alternatively, viral vectors, e.g., Adeno-associated virus, can be used to deliver the mutated gene to a stem cell, or may be used to target specific cells of a fully developed animal (32,33).

Antibodies and Recombinant Expression of Polypeptides

When the mutated gene product is a polypeptide, e.g. the 209 mutation, it can be used to prepare antisera and monoclonal antibodies using, for example, the method of Kohler and Milstein (34). Such monoclonal antibodies could

then form the basis of a diagnostic test, or may even be useful in therapies directed toward inhibiting the action of the mutant protein in a patient with Parkinson's disease.

5 Mutant polypeptides can also be used to immunize an animal for the production of polyclonal antiserum (35). For example, a recombinantly produced fragment of a variant polypeptide can be injected into a mouse along with an adjuvant so as to generate an immune response. Murine immunoglobulins which specifically bind the recombinant
10 fragment can be harvested from the immunized mouse as an antiserum, and may be further purified by affinity chromatography or other means. Additionally, spleen cells are harvested from the mouse and fused to myeloma cells to produce a bank of antibody-secreting hybridoma cells, which
15 can then be screened for clones that secrete immunoglobulins which bind the recombinantly produced fragment with an increased affinity. More specifically, immunoglobulins that selectively bind to the variant polypeptides but poorly or not at all to wild-type polypeptides are selected, either by
20 pre-absorption with wild-type proteins or by screening of hybridoma cell lines for specific idiotypes that bind the variant but not wild-type.

 These antibodies can be used to screen protein and tissue samples for the presence of mutated proteins. A
25 colored enzymatic reaction occurs when the specific antibody remains bound to its target protein, in situ, after thorough washing, as directed by established protocols.

Gene expression

The nucleic acid sequences of the present invention will be capable of expressing the desired mutant or normal polypeptides in an appropriate host cell. For expression in host cells, the DNA sequences of the present invention will be operably linked to, i.e., positioned to ensure the functioning of, an expression control sequence. For example, such polynucleotides can include a promoter, a transcription termination site (polyadenylation site in eukaryotic expression hosts), a ribosome binding site, and, optionally, an enhancer for use in eukaryotic expression hosts. In addition, the DNA sequence of the present invention may also be fused such that the reading frame is conserved to an appropriate signal sequence to facilitate export of the encoded protein across the cell membrane.

Expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. A variety of suitable expression vectors are disclosed in Sambrook et al. (13). Commonly, expression vectors will contain selection markers, e.g., tetracycline resistance or hygromycin resistance, to permit detection and/or selection of those cells transformed with the desired DNA sequences.

E. coli is one prokaryotic host that is particularly useful for cloning and expression of the DNA sequences of the present invention because of the wide variety of available expression systems. Vectors suitable for use in E. coli are

known and are commercially available, i.e. pBR322 (13),
pBLUESCRIPT (Stratagene), etc. Also, a variety of different
types of expression systems may be used, including plasmids,
cosmids, bacteriophage lambda, etc. Other microbial hosts
5 suitable for use include bacilli, such as *Bacillus subtilis*,
and other enterobacteriaceae, such as *Salmonella*, *Serratia*,
and various *Pseudomonas* species. Expression vectors for use
in prokaryotic host cells will typically contain expression
control sequences compatible with the host cell (e.g., an
10 origin of replication). In addition, any of a variety of
well-known promoters may be used, such as the lactose
promoter system, a tryptophan (Trp) promoter system, a
beta-lactamase promoter system, or a promoter system from
phage lambda. A promoter may optionally contain an operator
15 sequence for regulatable gene expression, and will have a
ribosome binding site sequence for the initiation of
translation.

In addition to microorganisms, mammalian tissue cell
culture may also be used to express and produce the
20 polypeptides of the present invention (36). Vectors for use
in eukaryotic cells are known and commercially available,
i.e. pcDNA3 (Invitrogen). Eukaryotic cells are actually
preferred, and a number of suitable host cell lines capable
of secreting intact human proteins have been developed in the
25 art, including CHO cells, COS cells, HeLa cells, myeloma cell
lines, Jurkat cells, etc. Promoters for use in eukaryotic
vectors may be cell-specific, or capable of being expressed

in a wide variety of cells, i.e. viral promoters.

Expression vectors of the present invention (e.g., comprising nucleic acid sequences encoding a mutant or normal polypeptide) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts.

Kits

The method lends itself readily to the formulation of test kits which can be utilized in diagnosis. Such a kit would comprise a carrier compartmentalized to receive in close confinement one or more containers wherein a first container may contain suitably labeled DNA probes. Other containers may contain reagents useful in the localization of the labeled probes, such as enzyme substrates. Still other containers may contain restriction enzymes (such as *Tsp45I*), buffers, etc., together with instructions for use.

DESCRIPTION OF THE INVENTION

Detailed Description of the Preferred Embodiments

The following laboratory procedures were used:

DNA samples were collected upon informed consent. High molecular weight genomic DNA was isolated from whole-blood

lysate by methods previously described (38). Genotyping was performed as previously described (39). Pairwise linkage analysis was performed using the MLINK program of the FASTLINK package (40-42). Allele frequencies were used as reported in the Genomic Data Base (<http://gdbwww.gdb.org>) and the Cooperative Human Linkage Consortium (CHLC) database (<http://www.chlc.org>). Multipoint analysis was performed using the LINKMAP program of the FASTLINK package. For the multipoint analysis allele frequencies were set to $1/n$ where n equals the number of alleles observed. In the two point analysis LOD scores were calculated for both the reported and the $1/n$ allele frequencies with minimal effect on the maximum LOD score observed. Simulations of multipoint analysis in a subset of the pedigree with different allele frequencies similarly indicated no significant effect on the scores calculated. Maximum LOD scores as shown were observed for the heterozygote and homozygote disease allele penetrance set to 0.99, which is similar to the PD allele penetrance previously reported ranging from 0.88 to 0.94 (3). All unaffected individuals used in the study were of age above the mean for onset of illness. Disease allele frequency was set to 0.0001. Distances and order of genetic markers were set as reported in the CHLC database. Overlapping three point analysis was performed for markers D4S2361, D4S1647, D4S421 and the PD locus. The 12 allele D4S2380 locus was not included because of prohibitive time run. Multipoint

analysis was performed on an IBM SP2 parallel computer and the SGI Challenge machine.

For mutation analysis genomic DNA was amplified with oligonucleotides (3): 5' GCTAATCAGCAATTTAAGGCTAG 3' (SEQ ID NO 2) and (13): 5' GATATGTTCTTAGATGCTCAG 3' (SEQ ID NO 3) of genbank ID: U46898, under standard PCR conditions. Sequence analysis was performed using the Perkin Elmer dye terminator cycle sequencing kit on an ABI 373 fluorescent sequencer (ABI, Foster City, CA). Restriction digestion was performed following the PCR with Tsp45 I according to manufacturer's protocol (New England Biolabs, Beverly, MA). The digested PCR products were electrophoresed on a 6% Visigel (Stratagene, La Jolla, CA), and visualized by ethidium bromide staining. Pedigree structure in Figure 2 has been slightly modified in order to protect patient confidentiality.

Total RNA was extracted from the lymphoblastoid cell line of an affected individual and first strand synthesis was performed by oligo dT priming (Gibco BRL, Gaithersburg, MD). Primers (1F) 5' ACGACAGTGTGGTGTAAAGG 3' (SEQ ID NO 9) and (13R) 5' AACATCTGTCAGCAGATCTC 3' (SEQ ID NO 10) corresponding to nucleotides 21-40 and 520-501 of genbank L08850 were used to amplify a product of 500 bp containing the mutation at nucleotide 209. PCR products were subjected to restriction digestion by Tsp45 I. The mutation at nt 209 creates a novel Tsp45 I site (Figure 1), so that the normal allele will be

restricted in 4 fragments of 249, 218, 24 and 9 bp, where the mutant allele will have 5 fragments of 249, 185, 33, 24 and 9 bp of size, as shown in Figure 3. Size standards used, were the 100 bp ladder (Gibco BRL, Gaithersburg, MD).

5

Example 1

In an effort to identify a genetic locus responsible for Parkinson's disease, we performed a genome scan in a large kindred of Italian descent with pathologically confirmed PD (Figure 5). The kindred originated in the town of Contursi in the Salerno province of Southern Italy (3). Some members emigrated to the United States, Germany and other countries. The extended family pedigree consists of 592 members with 60 individuals affected by PD. The average age of onset for the illness in this pedigree (Figure 5) has been shown to be 46 ± 13 years. One hundred and forty genetic markers were typed in this pedigree at an average spacing of about 20 cm. Genetic markers at the cytogenetic location 4q21-q23 were the only ones to show linkage to the disease phenotype with a $Z_{\max}=6.00$ at $\theta=0.00$ for marker D4S2380I (see Table 1).

20

Table 1. Two point LOD scores between chromosome 4q markers and the PD locus

	Locus	Two-point LOD scores at recombination fractions of:							Z_{\max}	θ_{\max}
		0.00	0.01	0.05	0.10	0.20	0.30	0.40		
5	D4S2361	-5.60	-0.83	0.30	0.54	0.43	0.21	0.06	0.55	0.12
10	D4S2380	6.00	5.90	5.30	4.60	3.00	1.50	0.50	6.00	0.00
	D4S1647	5.22	5.07	4.47	3.71	2.26	1.05	0.30	5.22	0.00
15	D4S421	-2.42	0.45	0.77	0.65	0.38	0.22	0.09	0.77	0.05

Recombinations between the disease phenotype and genetic markers were observed in the proximal region for marker **D4S2361** and in the distal region for marker **D4S421**. Genetic markers **D4S2380** and **D4S1647** showed no obligate recombination events in the affected individuals.

Multipoint LOD score analysis between markers **D4S2361**-13cM-**D4S1647**-3cM-**D4S421** and the disease locus places the PD gene between markers **D4S2361** and **D4S421** at a recombination distance of 0.00 cM from marker **D4S1647** with a $Z_{\max}=6.04$ (Figure 6). This location is favored from the alternative genetic intervals by a difference in the LOD score of greater than three LOD units.

Although expansions of unstable trinucleotide repeats are found in a number of human neurodegenerative conditions, there is no evidence for an association of a CAG trinucleotide repeat expansion in families with PD (43). In

addition, genetic linkage studies in other families with PD-like illnesses do not support the involvement of several candidate genes (glutathione peroxidase, tyrosine hydroxylase, brain-derived neurotrophic factor, catalase, amyloid precursor protein, CuZn superoxide dismutase and debrisoquinone 4-hydroxylase) in the etiology of the disorder (44). Genes previously mapped in the general region of linkage include the loci for alcohol dehydrogenase, formaldehyde dehydrogenase, synuclein, UDP-N-acetylglycosamine phosphotransferase and others.

Our localization of a PD susceptibility gene represents the first genetic locus linked to PD. Other distinct clinicopathological entities associated with parksonian features are probably linked to other genetic loci. For example, Wilhelmsen-Lynch disease (disinhibition-dementia-parkinsonian-amyotrophy complex) is linked to the 17q21-q22 chromosomal region (45). If the pathogenesis of diseases affecting the nigrostriatal pathway includes environmental influences, then a range of mutations affecting vulnerable sites in the electron transport chain or enzyme polymorphisms influencing neurotoxin metabolism may vary the penetrance of PD by altering an individual's resistance to exogenous or endogenous agents. However, our finding of a highly penetrant genetic locus linked to PD suggested that abnormalities of a single gene may be sufficient to cause Parkinson's disease.

Example 2

In an effort to identify a specific gene between markers D4S2361 and D4S421 that is associated with predisposition to Parkinson's disease, we conducted sequence analysis of candidate genes in this region.

Alpha synuclein, a presynaptic nerve terminal protein, was originally identified as the precursor protein for the NAC peptide, a non beta amyloid component of Alzheimer's disease (AD) amyloid plaques (4). The human alpha synuclein gene was previously mapped in the 4q21-q22 region (5). We refined the mapping, and determined that the alpha synuclein gene is located within the non-excluded region harboring the PD gene in the Italian kindred. Thus alpha synuclein represented an excellent candidate locus for PD.

Sequence analysis of the fourth exon of the alpha synuclein gene revealed a single base pair G209A change from the published sequence of the gene (GenBank ID L08850), which results in an Ala53Thr substitution and the creation of a novel Tsp45 I restriction site (Figure 1). Mutation analysis for the G209A change in the Italian kindred shows complete segregation with the PD phenotype with exception of individual 30 (Figure 2), who is affected but not carrying this mutation. This individual apparently inherited a different PD mutation from his father, as we have shown that

he shares a genetic haplotype with his unaffected maternal uncle, individual 3, for genetic markers in the PD linkage region.

The frequency of this variation was studied in two general population samples, one consisting of 120 chromosomes of the parents of the CEPH reference families, and the other consisting of 194 chromosomes of unrelated individuals from the blood bank in Salerno, Italy, a city near the town from which the family originated. Of these 314 general population chromosomes none was found to carry the G209A mutation. Fifty two patients of Italian descent with sporadic PD were also screened for the mutation (Figure 2), along with 5 probands from previously unpublished Greek families with PD. The Ala53Thr change was found to be present in three of the Greek kindreds and it segregated with the PD phenotype. In those three Greek kindreds it is worth noting that the age of onset for the disease is relatively early, ranging from the mid 30's to the mid 50's. Extended haplotype analysis of the Greek kindreds and the Italian PD family suggests that the mutations arose independently on different ancestral chromosomes. The finding of the Ala53Thr substitution in four independent PD families and its absence from 314 control chromosomes provides the strongest genetic evidence that this mutation in the human alpha synuclein gene is causative for the PD phenotype in these families.

We have also demonstrated by RT PCR that the mutant allele is transcribed in the lymphoblast cell line of an affected individual from the Italian kindred (Figure 3) (7). Thus, it is reasonable to assume that the mutant protein is indeed expressed.

Example 3.

Since homologous genes that are related to the alpha synuclein protein have been identified in other species, it seemed reasonable to assume that homologues of alpha synuclein would exist in humans as well. In fact, human beta synuclein has previously been described (46), and is approximately 60% similar to alpha synuclein at the protein level.

We set out to identify other related homologues by searching various databases for homologous genes and proteins. Protein sequence databases searched included the NR (non-redundant) and "month" databases of Genbank and Swiss Prot. Nucleotide databases included NR, month, dbstf, GSS (Genome Sequence Service) and EPD (eukaryotic Promoter Database). Several human clones were identified and characterized as alpha, beta and gamma clones as shown in Figure 7. Potential gamma clones were identified on the basis of homology to known rat and mouse sequences. Although gamma synuclein has been identified in species other than

human, this is the first identification of the corresponding gamma synuclein from humans.

Using two primers sets designed from known database sequences (5'ATGTCTTCAAGAAGGGCTTC3'; 5'CCTTGGTCTTCTCAGCTGCT3' and 5'AGCGTGGATGACCTGAAGAG3'; 5'AGCACAGGTGGACAGGCCAAG3'), we have isolated two BAC clones, 139A20 and 174P13, from a Genome System commercial Bacterial Artificial Chromosome library (St. Louis, MO) which contain the human beta and gamma synuclein genes, respectively. The beta gene contained one clone 139A20 has been sequenced as shown in Figure 8 (SEQ ID NO 11), which contains all coding exon sequences and some additional non-coding intronic sequence. The gamma clone 174P13 has been sequenced and is available in GenBank: accession number AF044311. Sequence from the 5' end is given in Figure 9 (SEQ ID NO 12), and sequence from the 3' end is given in Figure 10 (SEQ ID NO 13). The human alpha synuclein gene has also been sequenced as shown in Figure 11, which provides the sequence of each separate exon region with some additional flanking intronic sequence for each exon. (SEQ ID NOS 14-19)

The three human homologues are highly conserved at the protein level. The alpha and beta human homologues have about 60.4% similarity. And the gamma homologue is about 38.3% and 32.8% similar to the alpha and beta homologues, respectively, based on the portion of the coding sequence

that we have obtained thus far. Thus, it is reasonable to presume that mutations in either the beta or gamma synuclein gene may also result in Parkinson's disease.

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Each of the following citations is herein incorporated by reference:

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49. This application is based on provisional application number 60/505,684 filed June 25, 1997 which is relied upon and hereby expressly incorporated by reference herein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Polymeropoulos, Mihael
Lavedan, Christian
Leroy, Elisabeth
Nussbaum, Robert
Johnson, William
Duvoisin, Roger

(ii) TITLE OF INVENTION: Cloning of a gene mutation for
Parkinson's disease

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: SPENCER & FRANK
(B) STREET: 1100 New York Ave. Suite 300 East
(C) CITY: Washington
(D) STATE: D.C.
(E) COUNTRY: USA
(F) ZIP: 20005-3955

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE: 25-JUN-1998
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Schneller, John W.
(B) REGISTRATION NUMBER: 26,031
(C) REFERENCE/DOCKET NUMBER: NIH 0082A

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (202)414-4000
(B) TELEFAX: (202)414-4040

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 216 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

5 (iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

10 (B) CLONE: alpha synuclein gene/ exon 4 region

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

20 GCTAATCAGC AATTTAAGGC TAGCTTGAGA CTTATGTCTT GAATTTGTTT TTGTAGGCTC 60
CAAACCAAG GAGGGAGTGG TGCATGGTGT GACAACAGGT AAGCTCCATT GTGCTTATAT 120
CAAAGATGAT ATNTAAAGTAT CTAGTGATTA GTGTGGCCCA GTATCAAGAT TCCTATGAA 181
25 ATTGTAACA ATCACTGAGC ATCTAAGAAC ATATC 216

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "primer #3"

(iii) HYPOTHETICAL: NO

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

45 GCTAATCAGC AATTTAGGCT AG 22

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "primer #13"

(iii) HYPOTHETICAL: NO

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

10

CTATACAAGA ATCTACGAGT C

21

(2) INFORMATION FOR SEQ ID NO:4:

15

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 140 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: not relevant

20

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

25

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
 (C) INDIVIDUAL ISOLATE: Swiss-Prot P37840

30

(vii) IMMEDIATE SOURCE:
 (B) CLONE: alpha synuclein protein

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
 1 5 10 15

40

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
 20 25 30

45

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45

Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
 50 55 60

50

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
 65 70 75 80

Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys

85 90 95

Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile
100 105 110

5 Leu Glu Asp Met Pro Val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro
115 120 125

10 Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
130 135 140

(2) INFORMATION FOR SEQ ID NO:5:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

20 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

25 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rattus norvegicus
(C) INDIVIDUAL ISOLATE: Swiss-Prot P37377

30 (vii) IMMEDIATE SOURCE:
(B) CLONE: alpha synuclein protein

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
1 5 10 15

40 Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
35 40 45

45 Val His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
50 55 60

50 Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
65 70 75 80

Thr Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Phe Val Lys
85 90 95

Lys Asp Gln Met Gly Lys Gly Glu Glu Gly Tyr Pro Gln Glu Gly Ile
 100 105 110

5 Leu Glu Asp Met Pro Val Asp Pro Ser Ser Glu Ala Tyr Glu Met Pro
 115 120 125

Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
 130 135 140

10 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 134 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

20 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

25 (vi) ORIGINAL SOURCE:

(A) ORGANISM: Bos taurus
 (C) INDIVIDUAL ISOLATE: Swiss-Prot P33567

(vii) IMMEDIATE SOURCE:

30 (B) CLONE: alpha synuclein protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

35 Met Asp Val Phe Met Lys Gly Leu Ser Met Ala Lys Glu Gly Val Val
 1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Thr Glu Ala Ala Glu Lys
 20 25 30

40 Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45

45 Val Gln Gly Val Ala Ser Val Ala Glu Lys Thr Lys Glu Gln Ala Ser
 50 55 60

His Leu Gly Gly Ala Val Phe Ser Gly Ala Gly Asn Ile Ala Ala Ala
 65 70 75 80

50 Thr Gly Leu Val Lys Lys Glu Glu Phe Pro Thr Asp Leu Lys Pro Glu
 85 90 95

Glu Val Ala Gln Glu Ala Ala Glu Glu Pro Leu Ile Glu Pro Leu Met

100 105 110
 Glu Pro Glu Gly Glu Ser Tyr Glu Glu Gln Pro Gln Glu Glu Tyr Gln
 115 120 125
 5
 Glu Tyr Glu Pro Glu Ala
 130

(2) INFORMATION FOR SEQ ID NO:7:

- 10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 142 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 15 (D) TOPOLOGY: not relevant

 (ii) MOLECULE TYPE: peptide

 (iii) HYPOTHETICAL: NO
 20 (iv) ANTI-SENSE: NO

 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Serinus canaria
 25 (C) INDIVIDUAL ISOLATE: genbank L33860

 (vii) IMMEDIATE SOURCE:
 (B) CLONE: alpha synuclein homologue

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

35 Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Val Val Ala
 1 5 10 15

 Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys Thr
 20 25 30
 40 Lys Glu Gly Val Leu Tyr Val Gly Ser Arg Thr Lys Glu Gly Val Val
 35 40 45

 His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Ser Asn
 50 55 60
 45 Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys Thr
 65 70 75 80

 Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Leu Val Lys Lys
 85 90 95
 50 Asp Gln Leu Ala Lys Gln Asn Glu Glu Gly Phe Leu Gln Glu Gly Met
 100 105 110

Val Asn Asn Thr Gly Ala Ala Val Asp Pro Asp Asn Glu Ala Tyr Glu
 115 120 125

5 Met Pro Pro Glu Glu Glu Tyr Gln Asp Tyr Glu Pro Glu Ala
 130 135 140

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 10 (A) LENGTH: 143 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: not relevant
- 15 (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- 20 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Torpedo californica
 (C) INDIVIDUAL ISOLATE: Swiss-Prot P37379
- 25 (vii) IMMEDIATE SOURCE:
 (B) CLONE: alpha synuclein homologue

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Asp Val Leu Lys Lys Gly Phe Ser Phe Ala Lys Glu Gly Val Val
 1 5 10 15

35 Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Gln Asp Ala Ala Glu Lys
 20 25 30

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 35 40 45

40 Met Tyr Val Gly Thr Lys Thr Lys Glu Gly Val Val Gln Ser Val Asn
 50 55 60

45 Thr Val Thr Glu Lys Thr Lys Glu Gln Ala Asn Val Val Gly Gly Ala
 65 70 75 80

Val Val Ala Gly Val Asn Thr Val Ala Ser Lys Thr Val Glu Gly Val
 85 90 95

50 Glu Asn Val Ala Ala Ala Ser Gly Val Val Lys Leu Asp Glu His Gly
 100 105 110

Arg Glu Ile Pro Ala Glu Gln Val Ala Glu Gly Lys Gln Thr Thr Gln

115

120

125

Glu Pro Leu Val Glu Ala Thr Glu Ala Thr Glu Glu Thr Gly Lys
130 135 140

5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

10

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15

(A) DESCRIPTION: /desc = "primer #1F"

(iii) HYPOTHETICAL: NO

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACGACAGTGT GTGTAAAGG

19

25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

35

(A) DESCRIPTION: /desc = "primer #13R"

(iii) HYPOTHETICAL: NO

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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20

45

(2) INFORMATION FOR SEQ ID NO:11

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 2809 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

5 (iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 139A20 HUMAN BETA SYNULEIN GENE

(vi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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10 CAAGTTTGCA AGGGGCCCCG GANAAAAANC GAGCAGTGGC CCTTCCCGCG TCCCCAGGGT
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25 GACAATAACA GCACCCGCTT CCCAGGGCTG GGGAAAAGTG AAGTGCAGCG GGGCAGGCAG
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5 ATGATCTGGC CGGGAACCAG AGGGCGGGGG CGGGGGAGAC TCCCAAGGCT TCTGCGGGAA
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ACCATCCCCG CCCCCCTAAT CCTGCCACCA GCTTGAACA CAAGCCACTT TGCCTCCCAT
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15 ATTCAACAAA TATCCATGCC ANANCCAGCC CTGTCCTTGA GCTTCCAGNT CCCTTTTCAGC
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ATTGGGGGAG CGGCGTGGAG AGCCAGCTCC CCAATCCATG CTGCCACTTC AACTGTGATT
CGGGGGAATT TCCCCCTTCA CCTCCATCCC ACTTCCAAGG CACTCCAAAT AAATAACTGA
20 ATTAGAAATT ATCCTTGTTT TGCCAACCCA CCCTAGCCTT CCCCCTCCA ACCCACCACAA
AGCTTACCAC TGTGGGAATT TGGGGGGCAT CCTGGCTGTC CTCACGAGTC CTGACCTTTT
CTGCCCACAG CCAGAGGAAG TGGCCCAGGA AGCTGCTGAA GAACCACTGA TTGAGCCCCCT
GATGGAGCCA GAAGGGGAGA GTTATGAGGA CCCACCCCAG GAGGAATATC AGGAGTATGA
GCCAGAGGCG TAGGGGCCCA GGAGAGCCCC CACCAGCAGC ACAATTCTGT CCCTGTCCCT
25 GCCCCGCCCC CCAGAGCCAG GGCTGTCCTT AGACTCCTTC TCCCCAATCA CGAGATCTTC
CTTCCGCTCT GAGGCAACCC CCTCGGAGCC TGTGTTAGTG TCTGTCCATC TGTCTGTCCT
ACCCGCCCCG GTCCAACCCC GGGGCATGGA CAGGGCCAGG GTTGCGGTCTG CGGCTGGGAG

CCTCGCCCCCT CCAGTGTGTC CTCCTCCCAT CCAGCGTCTG CGCG

(2) INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISTICS

5 (A) LENGTH: 223 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

10 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 174P13 HUMAN GAMMA SYNULEIN GENE, 5' END

(vi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

15 AGGGAGATCC AGCTCCGTCC TGCCTGCAGC AGCACAACCC TGCACACCCA CCATGGATGT
CTTCAAGAAG GGCTTCTCCA TCGCCAAGGA GGGNGTGGTG GGTGCGGTGG AAAAGACCAA
GCAGGGGGTG ACGGAAGCAG CTGAGAAGAC CAAGGAGGGG GTCATGTATG TGGGATTACA
TTTTTTTTTT AAAGAAAGAA TAAATTAATT GTGATTAAAG TTG

20 (2) INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 677

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

25 (D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) IMMEDIATE SOURCE:

(A) CLONE: BAC clone 174P13 HUMAN GAMMA SYNULEIN GENE, 3' END

(vi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
5  TTTTTTNAGG GGGGAAAACA GGGAATANAA AAANANGGGG GGGGGTTTTT NNGGGGGGGG
   GGGGAAAANG GTTNGGGGGN NAACCNAAAN AAANNCCNAN GGGGGGGGNN ANTNAANTTT
   TGGGAACCCA AAGCCCNAGG AGGATTTTTN GTNAANAACG TNACCTCNAG TGGGNCGAGG
   AAGACCAAGG AAANGCCCAA CNCGGTTGAN CGAGGCTGTG GTGAACANCG TNCAACNCTG
   TGGCCNCCAA NANC GTGAG GNGGCGGAGA ACATCSCGGT CACCTCCGGG GTGGTGCGCM
10 AGGAGGACTT GAGGCCATCT KCCCCCMAC AGGAGGGTGT GGCATCCMAA GARAAAGAGG
   AAGTGGCAGA GGAGGCCAG AGTGGGGGAR ACTAGAGGGC TACAGGCCAG CGTGGATGAC
   CTGAAGAGCG CTCCTCTGCC TTGGACACCA TCCCCTCCTA GCACAAGGAG TGCCCGCCTT
   GAGTGACATG CGGCTGCCCA CGCTCCTGCC CTCGTCTTCC TGGCCACCCT TGGCCTGTCC
   ACCTGTGCTG CTGCACCAAC CTCACTGCCC TCCCTCGGCC CCACCCACCC TCTGGTCCTT
15 CTGACCCAC TTATGCTGCT GTGAATTTTT TTTTAAATG ATTCAAATA AACTTGAGC
   CCACTCCAAA AAAAAAA
```

(2) INFORMATION FOR SEQ ID NO:14

20 (i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1181 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

25 (ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exons 1 and 2 plus
flanking intron sequences

(viii) POSITION IN GENOME:

5 (A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AATTTTCAGCG ATGCGAGGGC AAAGCGCTCT CGGCGGTGCG GTGTGAGCCA CCTCCCGGCG
CTGCCTGTCT CCTCCAGCAG CTCCTCAAGG GATAGGCTCT GCCCTTGGTG GTCGACCCTC
10 AGGCCCTCGN TCTCCAGGN CGACTCTGAC GAGGGGTAGG GGGTGGTCCC CNGGAGGACC
CAGAGGAAAG GCNNGGACAA GAAGGGAGGG GAAGGGGAAA GAGGAAGAGG CATCATCCCT
AGCCCAACCG CTCCCGATCT CCACAAGAGT GCTCGTGACC CTAAACTTAA CGTGAGGCGC
AAAAGCGCCC CAACCTTTTC CCGCCTTGNN CCAGGCAGGC GGCTGGAGTT GATGGCTCAC
CCCGCGCCCC CTGCCCCATC CCCATCCGAG ATAGGGACGA GGAGCACGCT GCAGGGAAAAG
15 CAGCGAGCGC CGGGAGAGGG GCGGGCAGAA GCGCTGACAA ATCAGCGGTG GGGGCGGAGA
GCCGAGGAGA AGGAGAAGGA GGAGGACTAG GAGGAGGAGG ACGGCGACGA CCAGAAGGGG
CCCAAGAGAG GGGGCGAGCG ACCGAGCGCC GCGACCGGAA GTGAGGTGCG TCGGGGCTCA
GCGCAGACCC CGGCCCCGCC CCTCCTGAGA GCGTCCTGGG CGCTCCCTCA CGCCTTGCCT
TCAAGCCTTC TGCCTTTCCA CCCTCGTGAG CGGAGAACTG GGAGTGGCCA TTCGACGACA
20 GGTAGCGGG TTTGCCTCCC ACTCCCCCAG CCTCGCGTCG CCGGCTCACA GCGGCCTCCT
CTGGGGACAG TCCCCCCCCG GTGCCCCCTC GCCCTTCCTG TCGCTCCTT TTCCTTCTTC
TTTCCTATTA AATATTATTT GGGAAATTGTT TAAATTTTTT TTTTAAAAAA AGAGAGAGGC
GNNGGAGGAGT CGGAGTTGTG GAGAAGCAGA GGGACTCAGG TAAGTACCTG TGGATCTAAA
CGGGNGTCTT TTGAAATCC TGGAGAACGC CGGATGGAGA CGAATGGTCG TGGGNACCGG
25 GAGGGGGTGG TGCTGCCATG AGGACCGCTG GGCCAGGTCT CTGGGAGGTG AGTACTTGTC
CTTTGGGGAG CTAAGGAAAG AGACTTGACC TGGCTTTCGT CCTGCTTCTG ATATTCCCTT
CTCCACAAGG GCTGAGAGNT TAGGCTGCTT CTCCGGGATC C

(2) INFORMATION FOR SEQ ID NO:15

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 536 base pairs

(B) TYPE: NUCLEIC ACID

5 (C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

10 (vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exon 3 plus flanking
intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

15 (B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTTAAAAGAG TCTCACA CTGAGGGTTT CTCATGATTT TTCAGTGTTT TTTGTTTATT
TTTCCCCGAA AGTTCTCATT CAAAGTGTAT TTTATGTTTT CCAGTGTGGT GTAAAGAAAT
TCATTAGCCA TGGATGTATT CATGAAAGGA CTTTCAAAGG CCAAGGAGGG AGTTGTGGCT
20 GCTGCTGAGA AAACCAAACA GGGTGTGGCA GAAGCAGCAG GAAAGACAAA AGAGGGTGTT
CTCTATGTAG GTAGGTAAAC CCCAAATGTC AGTTTGTTGC TTGTTTCATGA GTGATGGGTT
AGGATAACAA TACTCTAAAT GCTGGTAGTT CTCTCTCTTG ATTCATTTTT GCATCATTGC
TTGTCAAAAA GGTGGACTGA GTCAGAGGTA TGTGTAGGTA GGTGAATGTG AACGTGTGTA
TNTGAGCTAA TAGTAAAAAT GCGACTGTTT GCTTTTCAGA TTTTAAATTT TGCCTAATAT
25 NTATGACTTN TTAATGAA TGTCTCTGTA CTACATAATT CTATNTCAGA GACAGT

(2) INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 650 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

5 (D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

10 (A) CLONE: human alpha synuclein gene/ exon 4 plus flanking
intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGCAGGTCA ACGGATCTGT CTCTAGTGCT GTACTTTTAA AGCTTCTACA GTTCTGAATT
CAAAATTATC TTCTCACTGG GCCCCGGTGT TATCTCATT C TTTTCTCTCC TCTGTAAGTT
GACATGTGAT GTGGGAACAA AGGGGATAAA GTCATTATTT TGTGCTAAAA TCGTAATTGG
AGAGGACCTC CTGTTAGCTG GGCTTTCTTC TATNTATTGT GGTGCTTAGG AGTTCCTTCT
20 TCTAGTTTTA GGATATATAT ATATATTTTT TCTTCCCTG AAGATATAAT AATATATATA
CTTCTGAAGA TTGAGATTTT TAAATTAGTT GTATTGAAAA CTAGCTAATC AGCAATTTAA
GGCTAGCTTG AGACTTATGT CTTGAATTTG TTTTGTAGG CTCCAAAACC AAGGAGGGAG
TGGTGCATGG TGTGGCAACA GGTAAGCTCC ATTGTGCTTA TATCAAAGAT GATATNTAAA
GTATCTAGTG ATTAGTGTGG CCCAGTATCA AGATTCCTAT TGAAATTGTA AAACAATCAC
25 TGAGCATCTA AGAACATATC AGTCTTATTG AAAGTGAATT CTTTATAAAG TATTTTTTAA
TAGGTAAATA TTGATTATAA ATAAAAAATA TACTTGCCAA GAATAATGAG

(2) INFORMATION FOR SEQ ID NO:17

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 504 base pairs

(B) TYPE: NUCLEIC ACID

5 (C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

10 (vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exon 5 plus flanking
intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

15 (B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATATCTTAGC CAAGATTCAA TGTTTGTTG AACCACTC ACTTGACATC TTGGTGGCTT
TTGTTTCTTC TGACCACTCA GTTATCTATG GCATGTGTAG ATACAGGTGT ATGGAANCGA
TGGCTAGTGG AAGTGGAATG ATTTTAAGTC ACTGTTATTC TACCACCCTT TAATCTGTTG
20 TTGCTCTTTA TTTGTACCAG TGGCTGAGAA GACCAAAGAG CAAGTGACAA ATGTTGGAGG
AGCAGTGGTG ACGGGTGTGA CAGCAGTAGC CCAGAAGACA GTGGAGGGAG CAGGGAGCAT
TGCAGCAGCC ACTGGCTTTG TCAAAAAGGA CCAGTTGGGC AAGGTATGGC TGTGTACGTT
TTGTGTTACA TTTATAAGCT GGTGAGATTA CGGTTTCAATTC TCATGTGAAG CCTGGAGGCA
GGAGCAAGAT ACTTACTGTG GGGAACGGCT ACCTGACCCT CCCCTTGTGA AAAAGTGCTA
25 CCTTTATATT GGTCTTGCTT GTTT

(2) INFORMATION FOR SEQ ID NO:18

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 727 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

5 (D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

10 (A) CLONE: human alpha synuclein gene/ exons 1 and 2 plus
flanking intron sequences

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AAAAGTTTAC ATACTTTGAG GTTGATAACC CATGTTGCCG CAATGTTTCC CCGGAGGCAT
TGTGGAGTTT AGAATGCCAG TAGTAATATT AAGGTGTGCC ATTTTCAAGA TCCGTGGCCA
ACATCCCTAT ATGTAAGATT TTTCCAAAAC ATGGTTCTGA TTTTAAAAAG TGAAAAATGC
TACTTCATCA TGTTCTTTTT GTGCTTCTTA CTTTAAATAT TAGAATGAAG AAGGAGCCCC
20 ACAGGAAGGA ATTCTGGAAG ATATGCCTGT GGATCCTGAC AATGAGGCTT ATGAAATGCC
TTCTGAGGTA GGAGTCCAAG CTGAATCTTT CTAACAAGAC AGTACCAAAA ACCTGTCATT
GTCACATTTT TCTTTCATTA GTGCTTAGTG AGAATCATTT GCTCTCTACA TGCTCATTA
GTGGACAAC T GCAAGTTAA GAATAGTTTT TACATTTTAA AAGGGTCCTT AAAAAAAAAAG
AGGAGGAGGA AGATGAAGAA GAGGAAGAAA GGATGTAAAA GAAATCATAT GTAGTCCACA
25 TAGCTTAATA TACNTACTAC TTGACCCTTT ACAGGAAAAG CTTTACTAAC CCCTGCATTA
GAGAATATAT TTTTTTGCAA AAACATTGAT TGTAATTTT AGTGTAAGT GGGGAGCCAT
TTCCTATCTC ATGGGCTGTC CAGTGCTGAT GCGTAATTGA AACTTATACT AACAGTGTGT

GCTGTCT

(2) INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISTICS

5 (A) LENGTH: 1596 base pairs

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: DOUBLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULAR TYPE: DNA (genomic)

10 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(A) CLONE: human alpha synuclein gene/ exon 7 plus flanking
intron sequences

15 (viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: 4

(B) MAP POSITION: 4q21-q22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTTTGATTTT TCTAATATTA GGAAGGGTAT CAAGACTACG AACCTGAAGC CTAAGAAATA
20 TCTTTGCTCC CAGTTTCTTG AGATCTGCTG ACAGATGTTT CATCCTGTAC AAGTGCTCAG
TTCCAATGTG CCCAGTCATG ACATTTCTCA AAGTTTTTAC AGTGTATCTC GAAGTCTTCC
ATCAGCAGTG ATTGAAGCAT CTGTACCTGC CCCCCTCAG CATTTCGGTG CTTCCCTTTC
ACTGAAGTGA ATACATGGTA GCAGGGTCTT TGTGTGCTGT GGATTTTGTG GCTTCAATCT
ACGATGTTAA AACAAATTAA AAACACCTAA GTGACTACCA CTTATTTCTA AATCCTCACT
25 ATTTTTTTGT TGCTGTTGTT CAGAAGTTGT TAGTGATTTG CTATCATATA TTATNAGATT
TTTAGGTGTC TTTTAATGAT ACTGTCTAAG AATAATGACG TATTGTGAAA TTTGTTAATA
TATATNATAC TTAATAATAT GTGAGCATGA AACTATGCAC CTATAATACT AAATATGAAA

TTTTACCATT TTGCGATGTG TTTTATTCAC TTGTGTTTGT ATATNAATGG TGAGAATTAA
AATAAAACGT TATCTCATTG CAAAAATATT TTATTTTAT CCCATCTCAC TTTAATAATA
AAAATCATGC TTATAAGCAA CATGAATTAA GAAGTGACAC AAAGGACAAA AATATAAAGT
TATTAATAGC CATTTGAAGA AGGAGGAATT TTAGAAGAGG TAGAGAAAAT GGAACATTAA
5 CCCTACACTC GGAATTCCCT GAAGCAACAC TGCCAGAAGT GTGTTTTGGT ATGCACTGGT
TCCTTAAGTG GCTGTGATTA ATTATTGAAA GTGGGGTGTT GAAGACCCCA ACTACTATTG
TAGAGTGGTC TATTTCTCCC TTCAATCCTG TCAATGTTTG CTTTACGTAT TTTGGGGAAC
TGTTGTTTGA TGTGTATGTG TTTATAATTG TTATACATTT TTAATTGAGC CTTTATTAA
CATATATTGT TATTTTGTG TCGAAATAAT TTTTGTAGTTA AAATCTATTT TGTCTGATAT
10 TGGTGTGAAT GCTGTACCTT TCTGACAATA AATAATATNC GACCATGAAT AAAAAAAAAA
AAAAAGTGGG TTCCCGGGAA CTAAGCAGTG TAGAAGATGA TTTTGAAGTAC ACCCTCCTTA
GAGAGCCATA AGACACATTA GCACATATTA GCACATTCAA GGCTCTGAGA GAATGTGGTT
AACTTTGTTT AACTCAGCAT TCCTCACTTT TTTTTTTTAA TCATCAGAAA TTCTCTCTCT
CTCTCTCTTT TTCTCTCGCT CTCTTTTTTT TTTTTTTTTT TTTTACAGGA AATGCCTTTA
15 AACATCGTTG GGAACACCA GAGTCACCTT AAAGGGAGNA TCAATTCTCT AGGACTGGAT
AAAAATTTCA TGGGCCTCCT TTAAAATGTT GCCCAAATAT ATGGAATTCT AGGGGTTTTT
CCNTAGGGGG AAGGGTTTTT TCTCTTTTCN GGGGAGGATC CTTTAAACNC CCCNGGGGGG
NGCCCGGAAA ATAAACTTGG NGGGGGGGNA AAACCTT

20

WHAT IS CLAIMED IS:

1. An isolated nucleic acid comprising a nucleotide sequence encoding a mutated human synuclein protein or homologue thereof.

5 2. The isolated nucleic acid of claim 1 wherein said mutated synuclein protein is selected from the group consisting of alpha, beta and gamma synuclein proteins.

 3. The isolated nucleic acid of claim 2 wherein said mutated
10 synuclein protein is the alpha synuclein protein.

 4. The isolated nucleic acid of claim 3 wherein said nucleotide sequence contains at least one mutation at base pair position 209.

15 5. The isolated nucleic acid of claim 4 wherein said mutation at position 209 is a change from guanine to adenine.

 6. The isolated nucleic acid of claim 5 having the sequence given
in SEQ ID NO. 1.

20

 7. An oligonucleotide complementary to a portion of the synuclein gene, wherein said portion comprises a mutation associated with predisposition to Parkinson's Disease.

25 8. The oligonucleotide of claim 7 wherein said mutation is at base pair position 209 in the synnuclein gene.

9. The oligonucleotide of claim 8 wherein said mutation is a change from guanine to adenine.

10. A vector comprising the isolated nucleic acid of claim 1.

5

11. A host cell comprising the vector of claim 10.

12. A method of affecting characteristics of Parkinson's Disease, comprising of expressing nucleic acids which are implicated in disease development in cultured cells through the use of expression vectors.

10

13. The method of claim 12 wherein the said nucleic acid is selected from the group consisting of alpha, beta, and gamma synuclein genes.

14. The method of claim 13 wherein the said nucleic acid encodes the mutated alpha synuclein protein.

15

15. The method in claim 14 wherein the said mutated alpha synuclein protein contains at least one mutation at base pair 209.

16. The method of claim 15 wherein said mutation at position 209 is a change from guanine to adenine.

20

17. An isolated human synuclein protein or peptide containing at least one mutation.

25

18. The isolated human synuclein protein or peptide of claim 17 wherein said protein or peptide is selected from the group consisting of

the human alpha, beta and gamma synuclein proteins or fragments thereof.

19. The isolated human synuclein protein or peptide of claim 18 having the sequence given in SEQ ID NO 5.

5

20. The isolated human synuclein protein or peptide of claim 19 wherein said protein or peptide is the alpha synuclein gene or a fragment thereof.

10

21. The isolated protein or peptide of claim 20, wherein said mutation is at amino acid position 53.

22. The isolated protein or peptide of claim 21, wherein said mutation is an alanine to threonine substitution.

15

23. An antibody specific for the protein or peptide of claim 17.

24. A method of detecting subjects at increased risk for Parkinson's Disease, comprising:

20

obtaining a sample comprising nucleic acids, proteins or tissues from the subjects, and

detecting in the nucleic acids, proteins or tissues the presence of a mutation which is associated with Parkinson's disease,

thereby identifying subjects at increased risk for the disease.

25

25. The method of claim 24 wherein said mutation is located on human chromosome four.

26. The method of claim 25 wherein said mutation is located in the alpha synuclein gene.

27. The method of claim 26 wherein said mutation causes an amino
5 acid substitution at position 53.

28. The method of claim 27 wherein said mutation causes an alanine to threonine substitution at position 53.

10 29. The method of claim 24 wherein said detecting step comprises combining a nucleotide probe which selectively hybridizes to a nucleic acid containing said mutation, and detecting the presence of hybridization.

15 30. The method of claim 29 wherein said nucleotide probe is an oligonucleotide complementary to a portion of the synuclein gene, wherein said portion comprises a mutation associated with predisposition to Parkinson's Disease.

20 31. The method of claim 30 wherein the mutation of said oligonucleotide is at base pair position 209 in the alpha synuclein gene.

32. The method of claim 31 wherein the mutation of said oligonucleotide is a change from guanine to adenine.

25

33. The method of claim 24 wherein said detecting step comprises amplifying a nucleic acid product comprising said mutation, and detecting

the presence of said mutation in the amplified product.

34. The method of claim 33 wherein said detecting step comprises selectively amplifying a nucleic acid product comprising said mutation,
5 and detecting the presence of amplification.

35. The method of claim 34 wherein said amplifying step comprises at least one annealing step whereby at least one oligonucleotide is annealed to said sample of nucleic acids.

10

36. The method of claim 35 wherein said amplifying step uses two oligonucleotides.

37. The method of claim 36 wherein said two oligonucleotides have
15 the sequences of SEQ ID NOS 2 and 3.

38. The method of claim 24 wherein said detecting step comprises detecting the presence or absence of a restriction endonuclease site as detected by enzymatic digest of said sample of nucleic acids.

20

39. The method of claim 38 wherein said restriction endonuclease site is recognized by *Tsp451*.

40. The method of claim 24 wherein said detecting step comprises
25 chain termination with a labeled dideoxynucleotide.

41. An oligonucleotide complementary to a nucleic acid sequence

which flanks a mutation in the synuclein gene that is associated with predisposition to Parkinson's disease, wherein said oligonucleotide may be used in diagnostic screens in the amplification of a nucleic acid sequence comprising said mutation.

5

42. The oligonucleotide of claim 41 having the sequence of SEQ ID NO 2.

10

43. The oligonucleotide of claim 41 having the sequence of SEQ ID NO 3.

44. The method of claim 24 wherein said detection step comprises identification of said mutations with an antibody.

15

45. The method of claim 44 wherein said antibody is directed against an isolated human synuclein protein or peptide containing at least one mutation.

20

46. The method of claim 45 wherein said isolated human synuclein protein or peptide is selected from a group consisting of the human alpha, beta, and gamma synuclein proteins or fragments thereof.

25

47. The method of claim 46 wherein said isolated human synuclein protein or peptide has the mutated sequence given in SEQ ID NO 5.

48. The method of claim 47 wherein said mutation is at amino acid position 53.

49. The method of claim 48 wherein said mutation is an alanine to threonine substitution

50. A diagnostic kit comprising the oligonucleotide of claim 41.

5

51. A diagnostic kit comprising the oligonucleotide of claim 42.

52. A diagnostic kit comprising the oligonucleotide of claim 43.

10

53. A diagnostic kit comprising the oligonucleotide of claim 7.

54. A diagnostic kit comprising the oligonucleotide of claim 8.

55. A diagnostic kit comprising the oligonucleotide of claim 9.

15

56. A diagnostic kit comprising the antibody of claim 23.

57. An isolated nucleic acid comprising a mutation in a human synuclein gene or homologue thereof.

20

58. The isolated nucleic acid of claim 57 wherein said synuclein gene is the alpha synuclein gene.

59. The isolated nucleic acid of claim 58 wherein said mutation occurs at base pair position 209.

25

60. The isolated nucleic acid of claim 59 wherein said mutation is

a change from guanine to adenine.

61. The isolated nucleic acid of claim 60 having the sequence given in SEQ ID NO 1.

5

62. A transgenic animal which expresses a mutated synuclein protein, wherein said animal may be used as an animal model for Parkinson's disease.

10 63. The transgenic animal of claim 62, wherein said mutated synuclein protein is an alpha synuclein protein.

64. A method of screening a compound for the ability to reverse the self-aggregation of synuclein proteins, comprising exposing an aggregate
15 of synuclein proteins to a test compound and observing whether or not the aggregate is dissolved.

65. The method of claim 64 wherein said test compound is a synuclein peptide.

20

66. The method of claim 65 wherein said peptide comprises a mutation.

67. The method of claim 64 wherein said test compound is an
25 antibody.

68. The method of claim 64, wherein said observing step comprises

Congo red staining, electron microscopy or CD spectrometry.

69. The method of claim 64 wherein said protein aggregate is located within an animal.

5

70. A method of screening a compound for the ability to inhibit the self-aggregation of synuclein proteins, comprising exposing a population of synuclein proteins to a test compound under conditions which promote self-aggregation in the absence of said compound and observing whether or
10 not self-aggregation of said proteins is inhibited.

71. The method of claim 70 wherein said test compound is a synuclein peptide.

15 72. The method of claim 71 wherein said peptide comprises a mutation.

73. The method of claim 70 wherein said test compound is an antibody.

20

74. The invention substantially as disclosed and described.





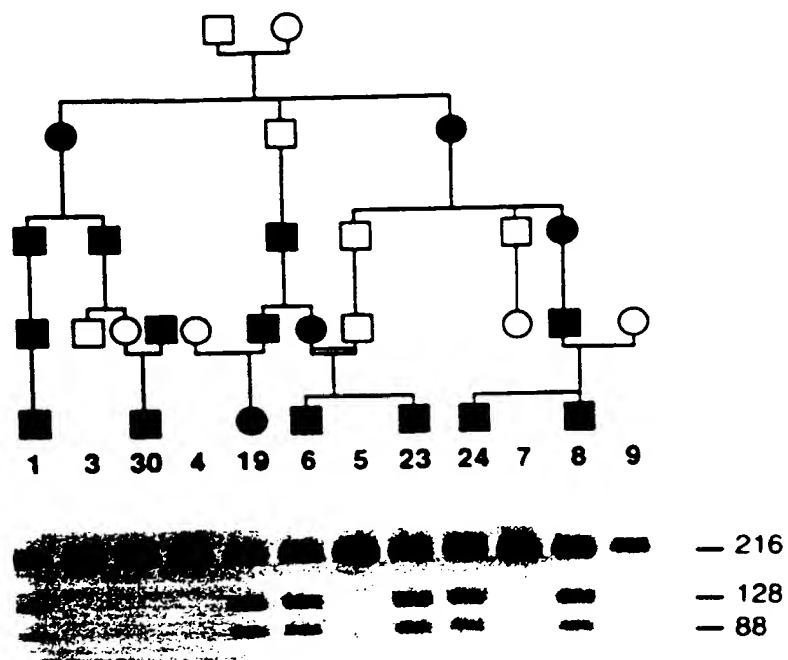


FIG. 2



Figure 4

1	MDVFMKGLSKAK	10	EGVVAAAEKTKQGVAAAGKT	20	EGVVAAAEKTKQGVAAAGKT	30	EGVVLY	Homo sapiens
1	MDVFMKGLSKAK		EGVVAAAEKTKQGVAAAGKT		EGVVAAAEKTKQGVAAAGKT		EGVVLY	Rattus norvegicus
1	MDVFMKGLSKAK		EGVVAAAEKTKQGVAAAGKT		EGVVAAAEKTKQGVAAAGKT		EGVVLY	Bos taurus
1	MDVFMKGLSKAK		EGVVAAAEKTKQGVAAAGKT		EGVVAAAEKTKQGVAAAGKT		EGVVLY	Serinus canaria
1	MDVLKKGFSAK		EGVVAAAEKTKQGVAAAGKT		EGVVAAAEKTKQGVAAAGKT		EGVMMY	Torpedo californica
40	VGSKTKEG	50	VHGVATV	60	AEKTKQGVAAAGKT	70	AEKTKQGVAAAGKT	Homo sapiens
40	VGSKTKEG		VHGVATV		AEKTKQGVAAAGKT		AEKTKQGVAAAGKT	Rattus norvegicus
40	VGSKTKEG		VHGVATV		AEKTKQGVAAAGKT		AEKTKQGVAAAGKT	Bos taurus
40	VGSRTKEG		VHGVATV		AEKTKQGVAAAGKT		AEKTKQGVAAAGKT	Serinus canaria
51	VGTKTKEG		VHGVATV		AEKTKQGVAAAGKT		AEKTKQGVAAAGKT	Torpedo californica
90	AATG	100	FVKKDQLGK-N	110	EEGAPQ-EEGI	120	MPVDPDNEAYEMPSP	Homo sapiens
90	AATG		FVKKDQLGK-N		EEGAPQ-EEGI		MPVDPDNEAYEMPSP	Rattus norvegicus
79	AATG		LKKKEEFP-T-DL		KEEVAQ-EEAE		PLMEPEGESYEEQP	Bos taurus
90	AATG		LKKDQLAKQN		EEGFLQ-EGM		VTGAAVDPDNEAYEMPSP	Serinus canaria
101	AASG		VVKKLDEHGR-EI		PAEQVABGKQTTEPLVB		ATEATE	Torpedo californica
130	EEGYQDYEP	140	EEA					Homo sapiens
130	EEGYQDYEP		EEA					Rattus norvegicus
124	QEEYQDYEP		EEA					Bos taurus
133	EEGYQDYEP		EEA					Serinus canaria
140	EEGYQDYEP		EEA					Torpedo californica



Figure 5

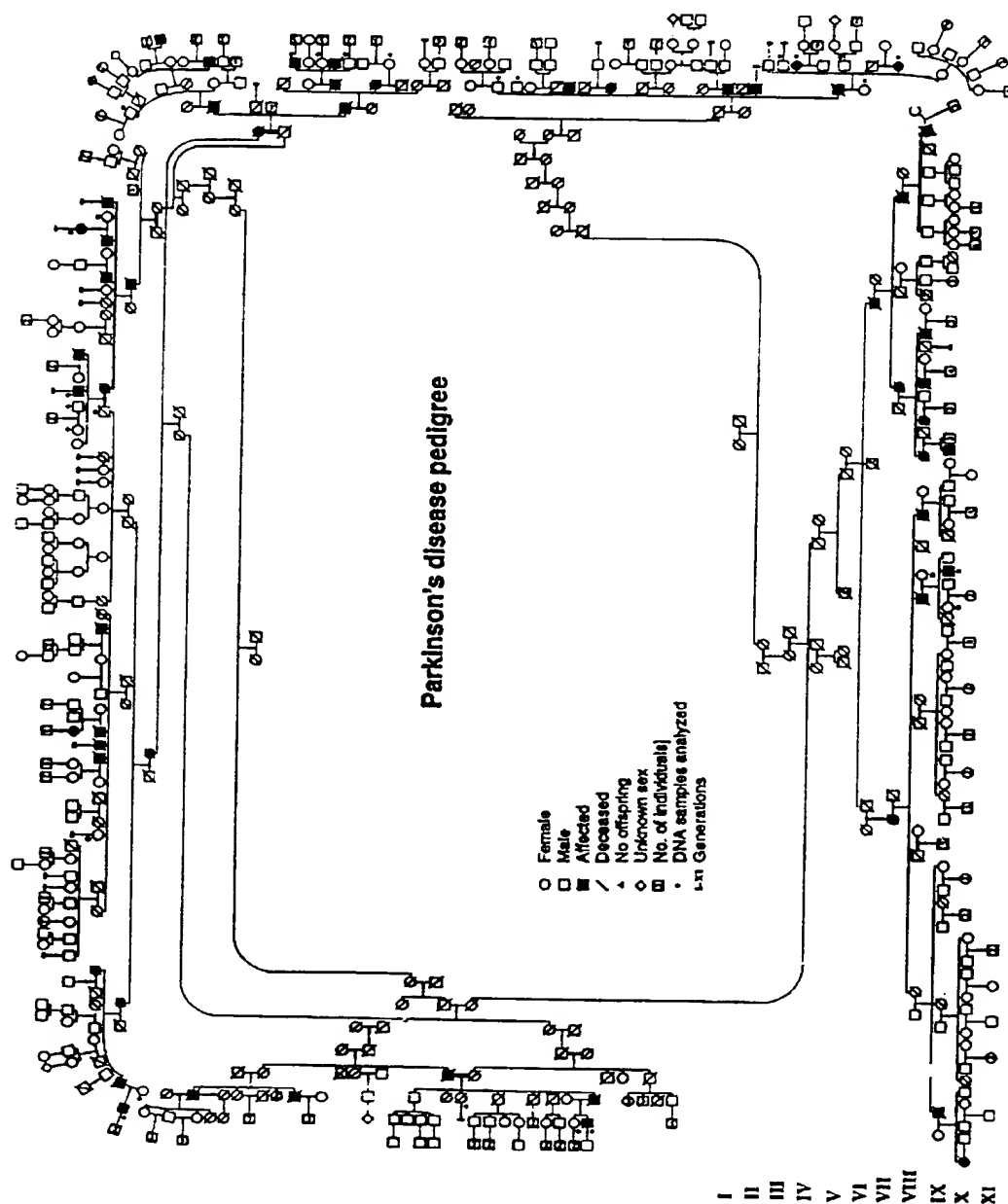
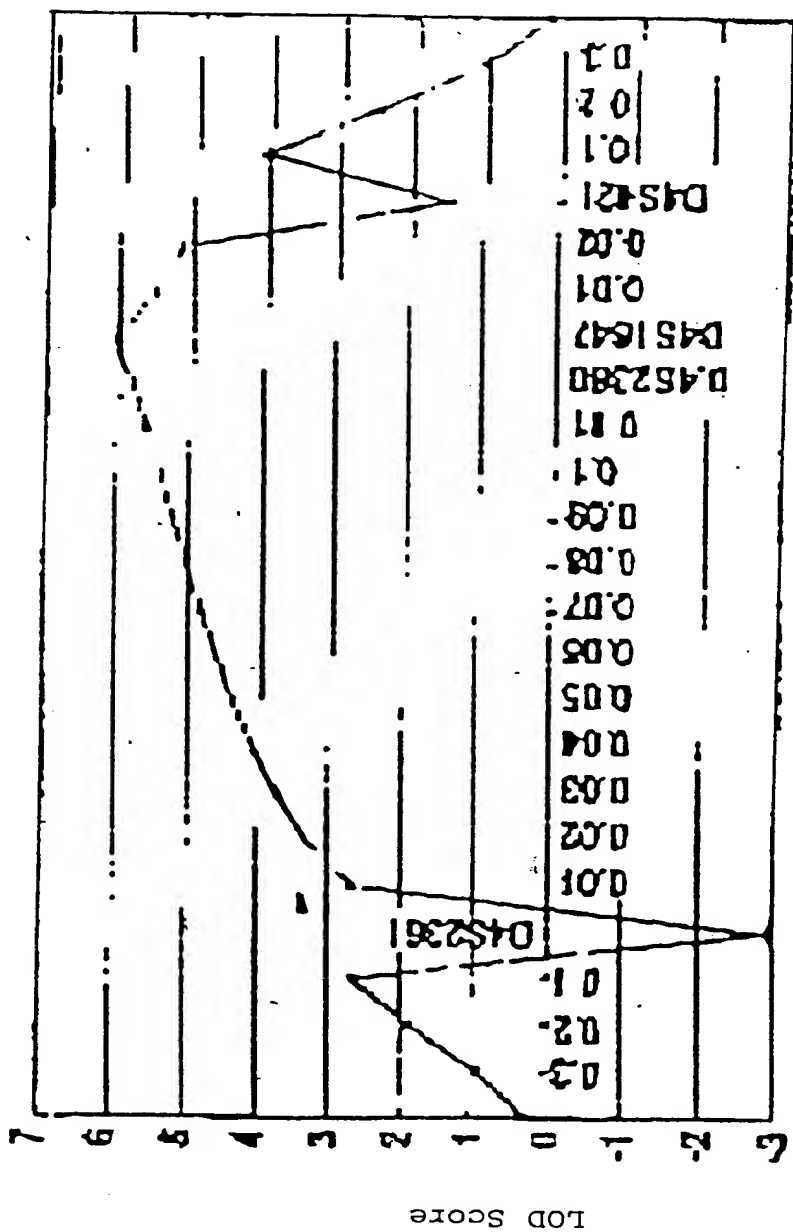




Figure 6





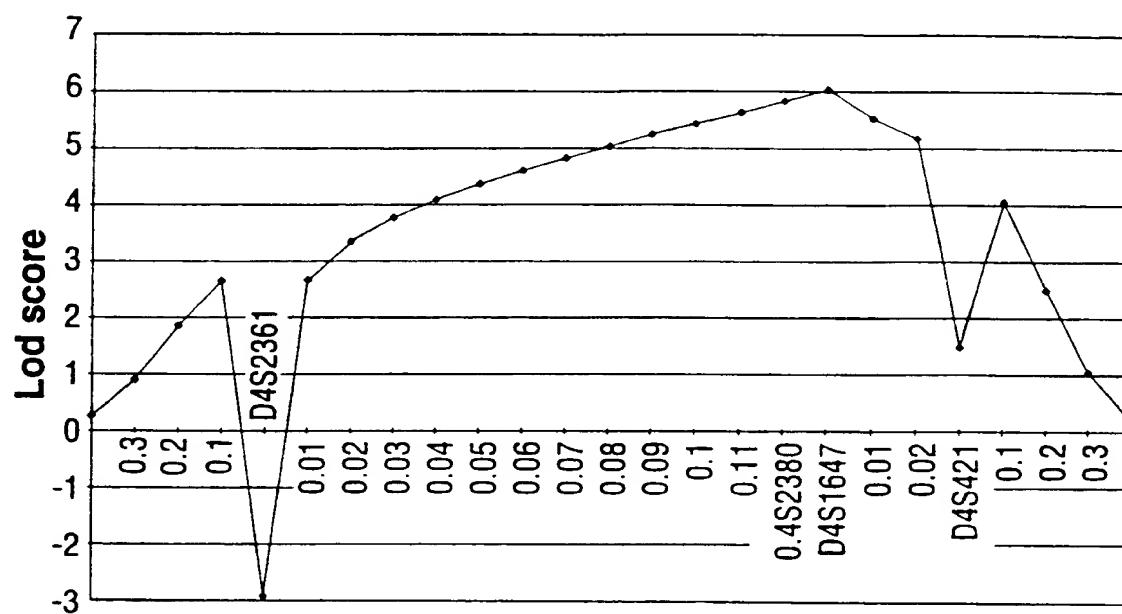


FIG. 6



Figure 7/1

clone	5'	3'	gene
109979	T84229	T88834	alpha
111088	T83410		alpha
111090	T83411	T81593	alpha
130048	R11819	(R19409)	alpha
135534	R31354	R32856	alpha
141248	R66863	R67383	alpha
145594	R78091	R77746	alpha
171906	H19290	H19291	beta
172284	H19556	H19474	beta
172749		H19685	beta
175546		H41126	beta
193174	H47503	H47504	alpha
210768	H66914	H66869	alpha
213616	H70324	H70325	alpha
235027	H62070		alpha
248153	N53829	N73325	alpha
24991	(T80528)	R39000	alpha
26298	R13508	(R20629)	alpha
265817	N28661	N21457	alpha
286628		N22757	alpha
27342		R37173	alpha
280344	(N50305)	N47094	alpha
290894		N72005	alpha
294142		N68597	alpha
307787	W21278		alpha
340835	W56712	W56757	alpha
340683	W55988	W56276	alpha
346647	W84390	W74638	alpha
346786	W79685	W79784	alpha
359349	AA010546	AA010547	alpha
364832	AA022809	AA022690	alpha
39915		R50455	beta
40764	R56327	R56245	alpha
45086	H08808	H08824	alpha
48807	H10267	H10213	alpha
49811	H29080	H28976	alpha
50202		H17862	beta
50470		H16811	beta
66473	R16018	R16119	alpha
667784	AA258686	AA258608	alpha
69907	T48654	T48655	alpha
72391	AA384097	AA283803	gamma
739009	AA421586		beta
739014	(AA42185)	AA421587	beta
771303		AA443638	gamma
2-4		L36675	alpha
2-5		L36674	alpha
c-01f06		F01363	alpha
c-1rb08	F03254	F06981	alpha
c-2td12	F08836	F11169	alpha
c-28f08	F03751	F07521	alpha
cDNA	S69965		beta
EST01420 (HRBAA27)	M79265		gamma
EST19193	AA317129		beta
EST22040	AA319774		alpha



Figure 7/2

EST26845	T28079		beta
EST31489	AA328063		alpha
EST68G11	W22518		gamma
F1-625D	R29481		alpha
GEN-129D09	D81090		beta
hbc590	T11070		alpha
HIBBA65	T08213	T08212	alpha
	HR70E3R	HR70E3F	alpha
HSNACP0		U46896-46901	alpha
KK1311	N83633		alpha
		D318839	alpha
		L08850	alpha
	T28735		alpha
	Z20502		alpha



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Figure 8

10 20 30 40 50 60 70
CCGCCGAGCCGCCGCTCCATCCCCAGCCCCGCCCGCATCCGGTTTGAAGGGGGCTGCAAGTTTGA 70
AGGGGCCCCGGGAXAAAAAXCAGAGCAGTGGCCCTTCCCGCTCCCCAGGGTTTCAAGGGACGCTAGGAXTX 140
TCCGCGGCCCTGGAGGTTGCACTGGGGAGTGGGGTGAGATGGGGGAAAGCGGGAGGGGGCTCAGGGTC 210
CAGAAGGGCXCXCGGCTC GGGAGTAGGGGGCATXTGCGTCCCGCGGGAGGGGCTGGGGTGAGAGTGC 280
GGGGCAGTGCACCGGTGCCCGTGTATCGCCCTCCCAGGCCAGGATGGACGTGTTTCATGAAGGGCC 350
360 370 380 390 400 410 420
TGTCATGGCCAAGGAGGGCGTTGTGGCAGCCCGGAGAAAACCAAGCAGGGGGTCACCGAGGCGGGCGGA 420
GAAGACCAAGGAGGGCGTCCCTACGTGCGTGGGCGXGGGGCGXGGGTTTCTGGGGCTGCAGGGCTGGGGG 490
TCCCCCTACAGTGTGGAGCTGGGGCGGGTCCCGGGAGGGGGGTTCTGGGCAAGATAATATXAXTCAGC 560
AGATGGGGCXAGGTCAAXCAGGGTCATAAGGGACATACCCAXCCCATAGAAXCCTGGGTCTGTATCCGGA 630
AATGGGGACACGGGGCGGGCTGTAGGTTGGGGGGTCCAXCTGAAAGGCCAGGGACCAXTGCAXTXATA 700
710 720 730 740 750 760 770
AAAXCACACAXCCTCCTTTTTCTTATCTTTTTTACCATTATTAATAGTTATCTGGTGTGAACACTTTCT 770
GTATGCCAAGTACTGGGTAATAATGTCATAACATCCATTTCCTCATGTAATGCTTCCGCCCATTTCTACAGG 840
TAAGGGAAACTGGGCTTCCATTGGTAGXTAAATTTTGAAGTTCAGAAAGGCTTGAATTGAATGTCAGTTC 910
AGCCAATTTCTTAGTGGTGGAAACCAACTGAGTTCCATCCGTGAAACGGGGACAATAACAGACCCGCTT 980
CCCAGGGCTGGGGAAAAGTGAAGTGCAGCGGGGAGGCAGAGGACTTGACACAGCACTGGCCCTCAGCCA 1050
1060 1070 1080 1090 1100 1110 1120
ACATCCACTAGAGGGGTGGGGTATCGCATCAGGTGGGAGAGAAGTCAACCCTTGACAGACAGAGGTGTGG 1120
GGCCAGTGCAGTGATAAGACGGGGGTTAATATGGGGTGCAGGTGTAGGATXTGGGGACCCAAGGAGG 1190
CAGTGCAGGGGGCAGGATGCCCACTCTGTAATCACCATGCTGTGCTGGAGTTTCTGTTCCCTCAGCGCAG 1260
AGTCCCTTAAATGTGCGCTTTTTCTXCCCIGCAGGAAGCAAGACCCGAGAAGGTGTGTTACAAGGTGTGG 1330
CTCAGGTACTAGCCCAGCCCTGGCACCAGCCCTTCTCTCAHTTAGGCGGATGATCTGGCCGGGAACCA 1400
1410 1420 1430 1440 1450 1460 1470
AGGGCGGGGGCGGGGGAGACTCCCAAGGCTTCTGCGGGAATGCTCCGTGGGGAGGGCAGGCCCTGGGATA 1470
CTACAAGGCAGGGCATCGGTGTTTCCCCCTGGCTCCCAAAACCCTTCTCAACCCCTCCCTGCTCCAGT 1540
GGCTGAAAAAACCAAGGAACAGGCCTCACATCTGGGAGGAGCTGTGTTCTCTGGGGCAGGGAACATCGCA 1610
GCAGCCACAGGACTGGTGAAGAGGGAGGAATCCCTACTGATCTGAAGGTAAGCGATCCTTCTGACCCGC 1680
ACATGCAGGCAAAAC 1750
1760 1770 1780 1790 1800 1810 1820
CCCCCTAATCCTGCCACCAGCTTGGAACACAAGCCACTTTGCTCCCATCCTGCXGGGCCGTGCTAGAC 1820
TCAGCTCAGAATGCATCTGAATAAXGGCGTGATGGGTGTGACGCTCCCGGTGATGGGGACCCAGACCTG 1890
GCTGTCTGCGTGTATCCTGCTTGCCAGCGTGACCATATGACTTCTGGCCACGTCTGCATGTGTCAATGA 1960
TTGTTCAATTCATTTCTTTTCAACAAATATCCATGCCAXXCCAGCCCTGTCTTGAGCTTCCAGXT 2030
CCCTTTTCCAGCCXAGGGGAGCXTGAGGGTTATTTTGGGGTCCCGATGCCAGCACAGAGCCTGACACAAA 2100
2110 2120 2130 2140 2150 2160 2170
GGATGAGGCATAAGCTGGTGAGTATCCAAATGGTGAAGTGTGGAGGXTGCCAGGCATTGGGGGAG 2170
CGGCGTGGAGAGCCAGCTCCCCAATCCATGCTGCCACTTCAACTGTGATTCGGGGGAATTTCCCCCTTCA 2240
CCTCCATCCCACTTCCAAGGCACTCCAAATAAATACTGAATTAGAAATTCCTTGTGTTTCCCAACCCA 2310
CCCTAGCCTTCCCCTCCAACCCACCCAAAGCTTACCAGTGTGGGAATTTGGGGGGCATCCTGGCTGTC 2380
CTCAGAGTCTGACCTTTTCTGCCACAGCCAGGAAGTGGCCAGGAAGCTGCTGAAGAACCACTGA 2450
2460 2470 2480 2490 2500 2510 2520
TTGAGCCCTGATGGAGCCAGAAGGGGAGAGTTATGAGGACCCACCCAGGAGGAATATCAGGAGTATGA 2520
GCCAGAGGCGTAGGGGGCCAGGAGAGCCCCACCAAGCAGCACAATTCTGCTCCCTGCTCCCTGCCCGCCCC 2590
CCAGAGCCAGGGCTGTCTTAGACTCCTTCTCCCAATCAGAGATCTTCTTCCGCTCTGAGGCAACCC 2660
CCTCGGAGCCTGTGTTAGTGTCTGTCCATCTGTCTGTCTACCCGCGCGCTCAACCCCGGGGCATGGA 2730
CAGGGCCAGGGTTGCGGTGCGGGCTGGGAGCCTGCCCTCCAGTGTGCTCCTCCATCCAGCGTCTG 2800
2810 2820 2830 2840 2850 2860 2870
CGCG 2804



Figure 9

10 20 30 40
AGGGAGATCCAGCTCCGTCCTGCCTGCAGCAGCACAAACCC 40
TGCACACCCACCATGGATGTCTTCAAGAAGGGCTTCTCCA 80
TCGCCAAGGAGGGXGTGGTGGGTGCGGTGGAAAAGACCAA 120
GCAGGGGGTGCAGGAAGCAGCTGAGAAGACCAAGGAGGGG 160
GTCATGTATGTGGGATTACATTTTTTTTTTAAAGAAAGAA 200
210 220 230 240
TAAATTAATTGTGATTAAAGTTG 223

Figure 10

10 20 30 40
TTTTTTXAGGGGGGAAAACAGGGAATAXAAAAAXAXGGGG 40
GGGGGTTTTTTXXGGGGGGGGGGGAAAAXGGTTXGGGGGX 80
XAACCXAAAXAAAXCCXAXGGGGGGGGXXAXTXAAXTTT 120
TGGGAACCCAAAGCCCXAGGAGGATTTTTXGTXAAXAACG 160
TXACCTCXAGTGGGXCGAGGAAGACCAAGGAAAXGCCCAA 200
210 220 230 240
CXCGGTTGAXCGAGGCTGTGGTGAACAXCGTXCAACXCTG 240
TGCCXCCAAXAXCGTGGAGGXGGGGGAGAACATCSCGGT 280
CACCTCCGGGGTGGTGC GCMAGGAGGACTTGAGGCCATCT 320
KCCCCC MACAGGAGGGTGTGGCATCCMAAGARAAAGAGG 360
AAGTGGCAGAGGAGGCCCAGAGTGGGGGARACTAGAGGGC 400
410 420 430 440
TACAGGCCAGCGTGGATGACCTGAAGAGCGCTCCTCTGCC 440
TTGGACACCATCCCCTCCTAGCACAAAGGAGTGCCCGCCTT 480
GAGTGACATGCGGCTGCCACGCTCCTGCCCTCGTCTTCC 520
TGGCCACCCTTGGCCTGTCCACCTGTGCTGCTGCACCAAC 560
CTCACTGCCCTCCCCTCGGCCCCACCCACCCTCTGGTCCTT 600
610 620 630 640
CTGACCCCACTTATGCTGCTGTGAATTTTTTTTTTAAATG 640
ATTCCAAATAAACTTGAGCCCACTCCAAAAA 677



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Figure 11/1

alpha-SYN exons 1-2

10 20 30 40
AATTTTCAGCGATGCGAGGGCAAAGCGCTCTCGGCGGTGCG 40
GTGTGAGCCACCTCCCGGCGCTGCCTGTCTCCTCCAGCAG 80
CTCCCCAAGGGATAGGCTCTGCCCTTGGTGGTTCGACCCTC 120
AGGCCCTCGNTCTCCAGGNCGACTCTGACGAGGGGTAGG 160
GGGTGGTCCCCNNGGAGGACCCAGAGGAAAGGCNNGGACAA 200

210 220 230 240
GAAGGGAGGGGAAGGGGAAGAGGAAGAGGCATCATCCCT 240
AGCCCAACCGCTCCCGATCTCCACAAGAGTGCTCGTGACC 280
CTAAACTTAACGTGAGGCGCAAAAGCGCCCCAACCTTTTC 320
CCGCCCTTGNCCAGGCAGGCGGCTGGAGTTGATGGCTCAC 360
CCCGCGCCCCCTGCCCATCCCCATCCGAGATAGGGACGA 400

410 420 430 440
GGAGCACGCTGCAGGGAAGCAGCGAGCGCCGGGAGAGGG 440
GCGGGCAGAAGCGCTGACAAATCAGCGGTGGGGGCGGAGA 480
GCCGAGGAGAAGGAGAAGGAGGAGGACTAGGAGGAGGAGG 520
ACGGCGACGACCAGAAGGGGCCCAAGAGAGGGGGCGAGCG 560
ACCGAGCGCCGCGACGCGAAGTGAGGTGCGTGCGGGCTCA 600

610 620 630 640
GCGCAGACCCCGGCCCGGCCCTCCTGAGAGCGTCCTGGG 640
CGCTCCCTCACGCCCTTGCCCTTCAAGCCTTCTGCCCTTTCCA 680
CCCTCGTGAGCGGAGAACTGGGAGTGGCCATTTCGACGACA 720
GGTTAGCGGGTTTGCCTCCCACTCCCCCAGCCTCGCGTCG 760
CCGGCTCACAGCGGCCCTCCTCTGGGGACAGTCCCCCCCCGG 800

810 820 830 840
GTGCCCTCCGCCCTTCTGTGCGCTCCTTTTCTTCTTC 840
TTTCCTATTAAATATTATTTGGGAATTGTTTAAATTTTTT 880
TTTTAAAAAAGAGAGAGGGCGNGGAGGAGTCGGAGTTGTG 920
GAGAAGCAGAGGGACTCAGGTAAGTACCTGTGGATCTAAA 960
CGGGNGTCTTTGGAAATCCTGGAGAACGCCGGATGGAGAC 1000

1010 1020 1030 1040
GAATGGTCGTGGGNACCGGGAGGGGGTGGTGCTGCCATGA 1040
GGACCGCTGGGCCAGGTCTCTGGGAGGTGAGTACTTGTCC 1080
TTTGGGAGCCTAAGGAAAGAGACTTGACCTGGCTTTTCGT 1120
CCTGCTTCTGATATTCCCTTCTCCACAAGGGCTGAGAGNT 1160
TAGGCTGCTTCTCCGGGATCC 1181



Figure 11/2

alpha-SYN exon 3

10 20 30 40
CTTAAAAGAGTCTCACACTTTGGAGGGTTTCTCATGATTT 40
TTCAGTGTTTTTGTATTATTTTCCCCGAAAGTTCTCATT 80
CAAAGTGATTTTATGTTTTCCAGTGTTGGTGTAAGAAAT 120
TCATTAGCCATGGATGTATTCATGAAAGGACTTTCAAAGG 160
CCAAGGAGGGAGTTGTGGCTGCTGCTGAGAAAACCAAACA 200
210 220 230 240
GGGTGTGGCAGAAGCAGCAGGAAAGACAAAAGAGGGTGTT 240
CTCTATGTAGGTAGGTAAACCCCAAATGTCAGTTTGGTGC 280
TTGTTTCATGAGTGATGGGTTAGGATAACAATACTCTAAAT 320
GCTGGTAGTTCTCTCTCTTGATTCAATTTTGCATCATTGC 360
TTGTCAAAAAGGTGGACTGAGTCAGAGGTATGTGTAGGTA 400
410 420 430 440
GGTGAATGTGAACGTGTGTATNTGAGCTAATAGTAAAAAT 440
GCGACTGTTTGCTTTTCAGATTTTAAATTTTGCCTAATAT 480
NTATGACTTNTTAAAATGAATGTTTCTGTACTACATAATT 520
CTATNTCAGAGACAGT 536



Figure 11/3

alpha-SYN exon 4

```

      10      20      30      40
CTGCAGGTCAACGGATCTGTCTCTAGTGCTGTACTTTTAA 40
AGCTTCTACAGTTCTGAATTCAAAATTATCTTCTCACTGG 80
GCCCCGGTGTTATCTCATTCTTTTTTCTCCTCTGTAAGTT 120
GACATGTGATGTGGGAACAAAGGGGATAAAGTCATTATTT 160
TGTGCTAAAATCGTAATTGGAGAGGACCTCCTGTTAGCTG 200
      210      220      230      240
GGCTTTCTTCTATNTATTGTGGTGGTTAGGAGTTCCTTCT 240
TCTAGTTTTTAGGATATATATATATATTTTTTTCTTTCCCT 280
GAAGATATAATAATATATATACTTCTGAAGATTGAGATTT 320
TTAAATTAGTTGTATTGAAAAGTAGCTAATCAGCAATTTA 360
AGGCTAGCTTGAGACTTATGTCTTGAATTTGTTTTTGTAG 400
      410      420      430      440
GCTCCAAAACCAAGGAGGGAGTGGTGCATGGTGTGGCAAC 440
AGGTAAGCTCCATTGTGCTTATATCAAAGATGATATNTAA 480
AGTATCTAGTGATTAGTGTGGCCAGTATCAAGATTCCTA 520
TGAAATTGTAAAACAATCACTGAGCATCTAAGAACATATC 560
AGTCTTATTGAAACTGAATTCTTTATAAAGTATTTTTTAAA 600
      610      620      630      640
TAGGTAAATATTGATTATAAATAAAAAATATACTTGCCAA 640
GAATAATGAG 650
```



Figure 11/4

alpha-SYN exon 5

10	20	30	40
ATATCTTAGCCAAGATTCAATGTTTGGTTGAACCACACTC	40		
ACTTGACATCTTGGTGGCTTTTGTCTTCTTGACCACTCA	80		
GTTATCTATGGCATGTGTAGATACAGGTGTATGGAANCGA	120		
TGGCTAGTGGAAGTGGAATGATTTTAAGTCACTGTTATTC	160		
TACCACCCTTTAATCTGTTGTGCTCTTTATTTGTACCAG	200		
210	220	230	240
TGGCTGAGAAGACCAAAGAGCAAGTGACAAATGTTGGAGG	240		
AGCAGTGGTGACGGGTGTGACAGCAGTAGCCCAGAAGACA	280		
GTGGAGGGAGCAGGGAGCATTGCAGCAGCCACTGGCTTTG	320		
TCAAAAAGGACCAGTTGGGCAAGGTATGGCTGTGTACGTT	360		
TTGTGTTACATTTATAAGCTGGTGAGATTACGGTTCATTT	400		
410	420	430	440
TCATGTGAAGCCTGGAGGCAGGAGCAAGATACTTACTGTG	440		
GGGAACGGCTACCTGACCCTCCCCTTGTGAAAAAGTGCTA	480		
CCTTTATATTGGTCTTGCTTGTTT	504		



Figure 11/5

alpha-SYN exon 6

10 20 30 40
AAAAGTTTACATACTTTGAGGTTGATAACCCATGTTGCCG 40
CAATGTTTCCCCGGAGGCATTGTGGAGTTTAGAATGCCAG 80
TAGTAATATTAAGGTGTGCCATTTTCAAGATCCGTGGCCA 120
ACATCCCTATATGTAAGATTTTCCAAAACATGGTTCTGA 160
TTTTTAAAAGTGAAAAATGCTACTTCATCATGTTCTTTTT 200
210 220 230 240
GTGCTTCTTACTTTTAAATATTAGAATGAAGAAGGAGCCCC 240
ACAGGAAGGAATTCTGGAAGATATGCCTGTGGATCCTGAC 280
AATGAGGCTTATGAAATGCCTTCTGAGGTAGGAGTCCAAG 320
CTGAATCTTTCTAACAAGACAGTACCAAAAACCTGTCATT 360
GTCACATTTCTCTTTTCATTAGTGCTTAGTGAGAATCATT 400
410 420 430 440
GCTCTCTACATGCTCATTACGTGGACAACCTTGCAAGTTAA 440
GAATAGTTTTTACATTTTTTAAAGGGTCCTTAAAAAAAAAAG 480
AGGAGGAGGAAGATGAAGAAGAGGAAGAAAGGATGTAAAA 520
GAAATCATATGTAGTCCACATAGCTTAATATACNTACTAC 560
TTGACCCTTTACAGGAAAAGCTTTACTAACCCCTGCATTA 600
610 620 630 640
GAGAATATATTTTTTTTGCAAAAACATTGATTGTAAATTTT 640
AGTGTAAGTGGGGAGCCATTTCTATCTCATTGGCTGTC 680
CAGTGCTGATGCGTAATTGAAACTTATACTAACAGTGTGT 720
GCTGTCT 727



alpha-SYN exon 7

10 20 30 40
TTTTGATTTTCTAATATTAGGAAGGGTATCAAGACTACG 40
AACCTGAAGCCTAAGAAATATCTTTGCTCCCAGTTTCTTG 80
AGATCTGCTGACAGATGTTCCATCCTGTACAAGTGCTCAG 120
TTCCAATGTGCCCAGTCATGACATTTCTCAAAGTTTTTAC 160
AGTGTATCTCGAAGTCTTCCATCAGCAGTGATTGAAGCAT 200
210 220 230 240
CTGTACCTGCCCCCACTCAGCATTTCGGTGCTTCCCTTTC 240
ACTGAAGTGAATACATGGTAGCAGGGTCTTTGTGTGCTGT 280
GGATTTTGTGGCTTCAATCTACGATGTTAAAACAAATTAA 320
AAACACCTAAGTGACTACCACCTATTTTCTAAATCCTCACT 360
ATTTTTTTGTGCTGTTGTTTCAGAAGTTGTTAGTGATTG 400
410 420 430 440
CTATCATATATTATNAGATTTTTAGGTGCTTTTAAATGAT 440
ACTGTCTAAGAATAATGACGTATTGTGAAATTTGTTAATA 480
TATATNATACTTAAAAATATGTGAGCATGAACTATGCAC 520
CTATAATACTAAATATGAAATTTTACCATTTTGCGATGTG 560
TTTTATTCACTTGTGTTTGTATATNAATGGTGAGAATTAA 600
610 620 630 640
AATAAACGTTATCTCATTGCAAAAATATTTTATTTTAT 640
CCCATCTCACTTTAATAATAAAAAATCATGCTTATAAGCAA 680
CATGAATTAAGAACTGACACAAAGGACAAAAATATAAAGT 720
TATTAATAGCCATTTGAAGAAGGAGGAATTTTAGAAGAGG 760
TAGAGAAAATGGAACATTAACCCTACACTCGGAATTCCT 800
810 820 830 840
GAAGCAACACTGCCAGAAGTGTGTTTTGGTATGCACTGGT 840
TCCTTAAGTGGCTGTGATTAATTATTGAAAGTGGGGTGTT 880
GAAGACCCCACTACTATTGTAGAGTGGTCTATTTCTCCC 920
TTCAATCCTGTCAATGTTTGCTTTACGTATTTTGGGGAAC 960
TGTGTTTGATGTGTATGTGTTTATAATTGTTATACATTT 1000
1010 1020 1030 1040
TTAATTGAGCCTTTTATTAACATATATTGTTATTTTGTG 1040
TCGAAATAATTTTTTAGTTAAAATCTATTTGTCTGATAT 1080
TGGTGTGAATGCTGTACCTTTCTGACAATAAATAATATNC 1120
GACCATGAATAAAAAAAAAAAAAAAGTGGGTTCCCGGGAA 1160
CTAAGCAGTGTAGAAGATGATTTTGACTACACCCTCCTTA 1200



Figure 11/7

alpha-SYN exon 7

1210 1220 1230 1240
GAGAGCCATAAGACACATTAGCACATATTAGCACATTCAA 1240
GGCTCTGAGAGAATGTGGTTAACTTTGTTTAACTCAGCAT 1280
TCCTCACTTTTTTTTTTTAATCATCAGAAATTCTCTCTCT 1320
CTCTCTCTTTTTCTCTCGCTCTCTTTTTTTTTTTTTTTTT 1360
TTTTACAGGAAATGCCTTTAAACATCGTTGGGAAC TACCA 1400
1410 1420 1430 1440
GAGTCACCTTAAAGGGAGNATCAATTCTCTAGGACTGGAT 1440
AAAAATTTTCATGGGCCTCCTTTAAAATGTTGCCCAAATAT 1480
ATGGAATTCTAGGGGTTTTTCCNTAGGGGGAAGGGTTTTT 1520
TCTCTTTTCNGGGGAGGATCCTTTTAACNCCCCNGGGGGG 1560
NGCCCGGAAAATAAACTTGGNGGGGGGGNAAAAC TT 1596



INTERNATIONAL SEARCH REPORT

Original Application No.

PCT/US 98/13071

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/47 C12N15/11 C07K16/18 A61K48/00
C12Q1/68 G01N33/68 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K C12Q G01N A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of the relevant passages	Relevant to claim No
X	SCHAPIRA A. H.: "Pathogenesis of Parkinson's disease." BAILLERES CLINICAL NEUROLOGY, vol. 6, no. 1, April 1997, pages 15-36, XP002083889	1-23, 57-61, 74
Y	see page 17, paragraph 2 see abstract	24-56, 62-73
Y	US 5 494 794 A (WALLACE DOUGLAS C) 27 February 1996 see the whole document	24-56, 62-73
	-/--	

☒ Further documents are listed in the continuation of box C

☒ Patent family members are listed in annex

Special categories of cited documents

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

10 November 1998

Date of mailing of the international search report

27/11/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Mandl, B

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/13071

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	JAKES R. ET AL.: "Identification of two distinct synucleins from human brain." FEBS LETTERS, vol. 345, 1994, pages 27-32, XP002078475 cited in the application & UEDA K. ET AL.: "Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease." PROC. NATL. ACAD. SCI. USA, vol. 90, 1993, pages 11282-11286, see figure 2 ---	1-74
A	CHEN X. ET AL: "The human NACP/alpha-synuclein gene: chromosome assignment to 4q21.3-q22 and TaqI RFLP analysis." GENOMICS, vol. 26, no. 2, 1995, pages 425-427, XP002083890 cited in the application ---	1-74
A	POLYMEROPOULOS M. H. ET AL.: "Mapping of a gene for Parkinson's disease to chromosome 4q21-q23." SCIENCE, vol. 274, 1996, pages 1197-1199, XP002083891 cited in the application see the whole document ---	1-74
A	MAROTEAUX L. AND SCHELLER R. H.: "The rat brain synucleins: family of proteins transiently associated with neuronal membrane." MOLECULAR BRAIN RESEARCH, vol. 11, 1991, pages 335-343, XP002083892 cited in the application see figure 1 ---	1-74
P,X	NUSSBAUM R. L. AND POLYMEROPOULOS M. H.: "Genetics of Parkinson's disease." HUMAN MOLECULAR GENETICS, vol. 6, no. 10, 1997, pages 1687-1691, XP002083893 see the whole document ---	1-74
P,X	GOEDERT M.: "The awakening of alpha-synuclein." NATURE, vol. 388, 17 July 1997, pages 232-233, XP002083894 see the whole document ---	1-74

-/--

INTERNATIONAL SEARCH REPORT

onal Application No
PCT/US 98/13071

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No
P.X	<p>POLYMEROPOULOS M. H. ET AL.: "Mutation in the alpha-synuclein gene identified in families with Parkinson's disease." SCIENCE, vol. 276, 27 June 1997, pages 2045-2047, XP002083895 see the whole document -----</p>	1-74

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/13071

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5494794 A	27-02-1996	WO 9409162 A	28-04-1994